AN ELECTROPHYSIOLOGICAL INVESTIGATION OF THE EFFECTS OF BEAK TRIMMING IN THE DOMESTIC FOWL (GALLUS GALLUS DOMESTICUS)

by

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DECLARATION

The work described in, and the composition of, this thesis is the author's own unaided work.

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ABSTRACT

Beak trimming, the amputation of the anterior part of the beak, is used to reduce feather pecking and cannibalism amongst intensively reared poultry. This practice has been criticized on the grounds that it may cause the bird to suffer pain.

The approach adopted in this thesis towards the study of pain perception in the chicken was to investigate the possibility of peripheral neural phenomena which may be related to acute and chronic pain sensation as a result of beak trimming.

A review of the literature pertaining to the peripheral neural basis of cutaneous sensation in birds revealed that whilst mechanoreceptors and thermoreceptors are known to be present in the avian beak, the evidence for nociceptors has not been conclusive.

Acute electrophysiological techniques were employed to study the primary afferent output from the beak, using a preparation developed for this purpose. Cutaneous nociceptors were discovered in the intact beak. An analysis of their stimulus response characteristics revealed many similarities with previously described mammalian cutaneous nociceptors. It was considered that the nociceptors would be activated during beak trimming, and would transmit nociceptive information to the central nervous system.

In the trimmed beak, the nociceptor population showed a reduced sensitivity to heat. They therefore did not provide a peripheral neural basis for hyperalgesia following beak trimming.

Abnormal spontaneous afferent discharges were recorded from the trimmed beak for up to 3 months following beak trimming. These discharges have many similarities to those resulting from peripheral nerve damage in mammals, and they may have had a similar origin in neuromas.

It was concluded that the acute and chronic peripheral neural consequences of beak trimming can be compared with processes which can give rise to acute and chronic pain sensations in man.

Further experiments are suggested to advance knowledge of the possibility of pain perception in the domestic fowl.

CONTENTS

Chapter 1.

INTRODUCTION AND LITERATURE REVIEW

1. INTRODUCTION AND LITERATURE REVIEW

I. INTRODUCTION

Intensively-reared poultry are susceptible to outbreaks of feather pecking and cannibalism (Brambell, 1965) which have economic and welfare implications for the poultry industry. For example, feather loss leads to increased heat loss and thus increased food uptake, and cannibalism can result in suffering and the death of many birds. Beak trimming, sometimes referred to as debeaking, is regarded by the poultry industry as one of the most economic and effective methods of reducing feather pecking and cannibalism. Beak trimming consists of the amputation of the anterior part of the beak, thereby making it difficult, if not impossible, for the bird to grasp the skin or feathers of other birds.

Several methods are used to effect this amputation. A typical method employs a device which consists of an electrically heated sharp upper blade which closes on an unheated lower blade. The beak of the unanaesthetized fowl is positioned between the upper and lower blades and the upper blade is closed on the beak, amputating the beak and cauterizing the stump in one operation.

In the United Kingdom, the Agriculture (Miscellaneous Provisions) Act (1968) defined beak trimming as "the removal from a bird of not more than one third of the beak, measured

from the tip towards the entrance to the nostrils". How well these recommendations are enforced is not clear. Little published information is available relating to the practice of beak trimming, for example the amount of beak removed, and the age at which beak trimming is carried out. No published surveys of beak trimming practice could be found. The only information found was unsatisfactory , eg. Sainsbury (1971) stated that "it is regrettable that all too often more than half the upper beak is removed". No substantial evidence was presented to support this statement. Data from a number of studies in the U.S.A. suggest that the amount of beak removed can vary enormously. For example, methods have been described which involve the removal of: one third of the upper and lower beak (Lonsdale, Vondell and Ringrose, 1957), one half of the upper and lower beak (Hargreaves and Champion, 1965), two thirds of the upper beak and one third of the lower beak (Andrade and Carson, 1969, 1975), two thirds of upper and lower beak (Lonsdale et al 1957; Andrade and Carson 1969, 1975), three quarters of the upper and lower beak (Hargreaves and Champion, 1965) and the whole beak (Hargreaves and Champion, 1965). In all these reports the proportion of the beak removed refers to the proportion of the distance between the tip of the beak and the anterior edge of the external nostrils. Again, there is no quantitative information available regarding the prevalence of each of these methods in the U.S.A. poultry industry.

The practice of beak trimming has been criticised by the Brambell Committee which was appointed to investigate the welfare of livestock kept under systems of intense husbandry. The basis for their objection was that beak trimming causes "severe pain" to the bird (Brambell, 1965). This conclusion by the Brambell Committee was based on the anatomical observation that "between the horn and bone (of the beak) is a thin layer of highly sensitive soft tissue, resembling the quick of the human nail. The hot knife blade used in debeaking cuts through this complex of horn, bone and sensitive tissue causing severe pain" (Brambell, 1965). This assertion, by the Brambell Committee, that beak trimming causes pain to the fowl has not been investigated despite the publication of the report, and forms the basis for the present investigation.

The International Association for the Study of Pain has defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (I.A.S.P., 1979). The approach adopted for the present investigation was to consider the first step in the process of nociception, the response of the peripheral nervous system to external stimuli. The purpose of this study is to determine if the peripheral nociceptive activity necessary for the experience of pain occurs during and after the process of debeaking.

The literature review that follows is intended to summarize present knowledge concerning the peripheral neural basis of sensation in the chicken beak.

Terminology

Throughout this thesis for convenience several domestic species of birds are referred to by their English names. Their scientific names are accordingly given below.

Chicken	Gallus gallus domesticus
Pigeon	Columba livia
Duck	Anas platyrhynchos
Goose	Anser anser

II. THE CHICKEN BEAK, ITS STRUCTURE AND INNERVATION

The chicken's beak plays a role in a wide range of activities. Is is used not only in feeding and drinking but also in exploratory activity, preening, nest making and turning incubating eggs, and displays and actions involving aggression, defence and courtship. As would be expected from an organ involved in so many interactions with the external environment, the beak has an extensive nerve supply, and sensory receptors which can detect and transmit information regarding the mechanical and thermal environment.

A. Gross Anatomy

The chicken beak, or rostrum, consists of an upper, maxillary, beak and a lower, mandibular, beak. The skeletal basis of the beak, (fig.1:1), has been described by Jollie (1957), Feduccia (1975), King and McLelland (1975) and Nickel, Schummer and Sieferle (1979). The articulations within the mandible have been described in detail by Hogg (1983).

The outer surface and part of the inner surface of the beak are covered with a keratinized thickening of the corneum of the epidermis, the rhamphotheca (Stettenheim, 1972). The rhamphotheca is hard and heavily cornified in the chicken, and in the pigeon, and it is sometimes referred to as the horny sheath (Nickel et al, 1973). In the duck and goose the

rhamphotheca is relatively soft and flexible and it is referred to as waxy skin or ceroma (Gottschaldt and Lausmann, 1974; Nickel et al, 1977). In the chicken and pigeon the beak is pointed and the horny sheath of the upper beak extends in a hook beyond that of the lower beak, (fig.1:2). The edges, or tomia, of both the upper and lower sheaths are sharp. In the duck and goose the beak is spoon shaped from the dorsal view, and it is covered by the ceroma except at the tip where the rhamphotheca forms a hard horny plate shaped like a finger nail (Nickel et al, 1977; Berkhoudt, 1980). In the chicken the waxy skin is confined to the base of the beak, and in the pigeon it forms swellings known as the cere. In the duck and goose, both filter feeding birds, there are horny lamellae arranged vertically along the edges of the upper and lower beak (Berkhoudt, 1977; Nickel et al, 1977). When the beak is closed these lamellae act as sieves, which retain small food particles taken up with the water. A detailed description of the structure and function of this filtering apparatus is given by Zweers, Gerritson and van Kranenburg-Voogd (1977).

B. Microanatomy

The histology of the chicken beak has been described comprehensively by Lucas and Stettenheim (1972), and most features are illustrated in fig.1:3. The bones of the beak are covered by dermis which is not differentiated into layers. Collagen and elastic fibres run in many directions and join the periosteum, holding the rhamphotheca to the bones. The dermis is heavily vascularised and contains many neve fibres and endings including encapsulated sensory receptor endings. Dermal papillae which protrude into the epidermis are found in the rhamphotheca and palate. The horny epidermis is thickest on the tomial edges and on the dorsal part of the upper beak, the culmen. It is thinnest on the inner, palatinal, surfaces. The epidermis is composed of two major layers, the superficial cornified layer or stratum corneum and the germinative layer, the stratum germinativum. The stratum germinativum consists of three layers; the basal layer, stratum basale, the intermediate layer, stratum intermedium and the transitional layer, stratum transitivum. The granular layer, stratum granulosum, and the clear layer, stratum lucidum, both found in mammalian epidermis, are absent from avian epidermis (Lucas and Stettenheim, 1972). The rhamphotheca continually grows towards the tip and the edges of the beak, where it is worn down with use.

C.Innervation

The innervation of the chicken beak has been described by Cords (1904), Kaupp (1918) and Haller (1974). More recent descriptions have been provided by Watanabe and Yasuda (1970), Baumel (1975), Nickel et al (1977) and Bubien-Waluszewska (1981). There is little disagreement over the main features, differences being primarily in the nomenclature applied to the various nerve branches. The terminology employed by Nickel et al (1977) based on the work of Schrader (1970), has been adopted in this study. The organization of the innervation is illustrated diagrammaically in fig.1:4 and is briefly summarized below.

The beak is innervated by branches of the three divisions of the fifth cranial nerve, nervus trigeminus. The three divisions are n. ophthalmicus, n. maxillaris and n. mandibularis. The upper beak is innervated by n. ethmoidalis, a branch of n. ophthalmicus, and n. palatinus major, a branch of n. maxillaris. N. ethmoidalis gives off a branch to the outer surface of the beak and another branch which innervates the mucosa of the rostral half of the palate and the tip of the beak. N. palatinus major innervates the posterior part of the palate.

The lower beak is innervated by three branches of n. mandibularis, namely r. angularis oris, n. sublingualis and n.

alveolaris mandibulae. R. angularis oris sends branches to the glands and mucosa of the palate medial to the angle of the mouth. N. sublingualis innervates the mucous membrane on the floor of the mouth rostral and lateral to the tongue. N. alveolaris mandibulae enters the foramen mandibulare, the entrance to the mandibular canal and runs along this canal. It innervates the skin of the region between the two rami of the lower beak, the wattles and the rhamphotheca.

III. CUTANEOUS SENSORY RECEPTORS IN BIRDS.

Birds have been shown to possess cutaneous mechanoreceptors, thermoreceptors and nociceptors. Some physiological aspects of the sensory innervation of the chicken beak have been described by Roumy and Leitner (1973). Most electrophysiological and anatomical investigations have been directed at other avian species, notably the pigeon, duck and goose. A review of the characteristics of avian cutaneous receptors now follows. Where possible, a correlation of response characteristics with morphologically distinct receptor structures will be made. Comparisons with mammalian sensory receptors will also be made where possible.

In all the literature cited in this review the electrophysiological technique employed by the respective authors was single unit dissection of primary afferent nerve fibres, except where otherwise stated.

A. CUTANEOUS MECHANORECEPTORS

1. Chicken

Roumy and Leitner (1973) and Skoglund (1960) have described some physiological responses of mechanoreceptors in the chicken. Roumy and Leitner (1973) recorded from the ophthalmic branch of the trigeminal nerve, which innervates the upper beak. They reported the presence of both rapidly adapting (RA) and slowly adapting (SA) mechanoreceptors. The RA mechanoreceptors were divided into two types, "vibration-sensitive" and "vibration-insensitive" (Roumy and Leitner, 1973). No further information on their functional characteristics was reported. Receptive fields of the mechanoreceptors were located on the dorsal and ventral surface of the beak, the upper part of the nostril and the base of the comb. The receptive fields of the SA mechanoreceptors were located on the posterior part of the nostrils. No further information on their characteristics was given by Roumy and Leitner (1973).

Skoglund (1960) recorded from the afferent nerve fibres of individual Herbst corpuscles. These corpuscles, which anatomically resemble the mammalian Pacinian corpuscles, were surgically exposed in the interosseous region between the tibia and fibula of the leg. The Herbst corpuscles responded to a vibratory stimulus with an in-phase discharge (stimulus

parameters not described).

2. Other Avian Species

(a) Rapidly adapting mechanoreceptors

The physiological characteristics of two types of RA mechanoreceptors have been described in the goose, duck and pigeon. Gottschaldt (1974a) has named these two types "Herbst units" and "Grandry units " respectively, these names referring to the receptor structure held to be responsible for the physiological response.

(i) Herbst Units

The physiological characteristics of Herbst units in the beak have been described by Gottschaldt (1974a,b), Gottschaldt and Lausmann (1974a) in the goose and Gregory (1973), Leitner, Roumy and Saxod (1973) and Leitner and Roumy (1974) in the duck. They have also been described in the tongue of the duck (Leitner and Roumy, 1974), in the wing of the duck (Dorward, 1970) and the pigeon (Horster, Shen and Schwartzkopff, 1983), and in the leg of the duck (Dorward and McIntyre, 1971) and the pigeon (Horster et al, 1983).

The distinguishing features of the Herbst units described by all these authors can be summarized in the following general terms. The units are not spontaneously active. They do not

respond during a maintained mechanical indentation of the integument, discharging a few impulses only at the onset and removal of such a stimulus. They are sensitive to moving mechanical stimuli, particularly a vibrating stimulus. The Herbst units display a consistent relationship to a vibrating, sinusoidal mechanical stimulus, discharging impulses at the same frequency as the stimulus, i.e. a 1:1 response, over a certain frequency range. This range is 40-1000 Hz for the Herbst units in the beak of the goose (Gottschaldt, 1974a,b) and duck (Leitner and Roumy, 1974; Leitner et al, 1973). Variations have been reported, e.g. Gregory (1973) reports a much lower value (1Hz) for the lowest frequency followed. The highest frequency followed by the Herbst units in the duck tongue (Leitner er al, 1973) and the pigeon leg and wing (Horster et al, 1983) is much lower, i.e. 300Hz, than that reported for the beak units.

The variation in the frequency-following capabilities between species and locations could be due to several factors, i.e. differences in experimental technique, species specific differences in the receptors, location-specific differences in the receptors, differences in the mechanical properties of the overlying and surrounding tissues, or even unavoidable sampling bias on the part of the investigators. With regard to the possibility of location-specific diffences none of the authors relate the properties of individual units to individual

receptive field locations so this point cannot be clarified. The possibility of sampling bias is raised by the observation of several authors that there was wide variation between individual Herbst units in their frequency-following capability.

Typically, above and below the range of frequencies followed in a 1:1 manner by any individual Herbst unit, the 1:1 relationship breaks down. Vibrations below the minimum 1:1 frequency result in more than one impulse discharged per vibration cycle, whilst vibrations above the maximum 1:1 frequency produce an impulse every second or third vibration cycle (Dorward, 1970; Gottschaldt, 1974a,b; Gregory, 1973; Dorward and McIntyre, 1971; Horster et al,1983)

The response of the Herbst units to a non-vibratory moving stimulus was investigated by Gregory (1973) in the duck beak and Gottschaldt (1974a,b) and Gottschaldt and Lausmann (1974a) in the goose beak, yielding mutually contradictory results. Gregory (1973), found that the response (impulse frequency) was linearly related to the logarithm of the stimulus velocity over a range of 0.1 to 70mm/sec. In contrast to this Gottschaldt (1974a,b) and Gottschaldt and Lausmann (1974a) found no relationship between the impulse frequency and the stimulus velocity. This discrepancy cannot be attributed to differences in experimental technique. It could perhaps reflect a species

difference in the receptors themselves. Another possibility is that Gregory (1973) could have confused two different types of unit. This possibility is raised by the fact that Gottschaldt (1974a,b) and Gottschaldt and Lausmann (1974a) described another type of RA mechanoreceptor which had a limited vibration sensitivity, only up to 200Hz, but was velocity sensitive. This type of RA mechanoreceptor is the Grandy unit of Gottschaldt (1974a,b) and is described in detail below. Gregory (1973) considered all the RA units he recorded from the duck beak as one group i.e. vibration-sensitive RA mechanoreceptors. The possibility that he may have lumped together two distinct types of RA mechanoreceptors is reinforced by the fact that Leitner and Roumy (1974a) described both Herbst and Grandy units in the same preparation as Gregory (1973). It is interesting in this context to note that Gregory (1973) reported a much lower minimum frequency followed by his sample of units, i.e. 1Hz, than the other reports on beak Herbst units ie. 40-50Hz. It is therefore possible that the lower frequency following units of Gregory's (1973) sample were in fact Grandy and not Herbst units.

The sizes of the receptive fields described for the Herbst units vary enormously, from 0.5mm. diameter (Gregory, 1973) up to the entire surface of the beak skin (Gottschaldt, 1974a,b). Dorward (1970), Dorward and McIntyre (1971), and Gottschaldt (1974a,b) all reported that some of their Herbst units were

excited by a vibratory mechanical stimulus applied anywhere on the wing, leg or beak respectively, or even on the metal baseplate supporting the animal. This phenomenon, and the range of receptive field sizes, is presumably due to the different locations of the actual receptor structures and the varying amounts of connective tissue separating the receptor from hard tissues e.g. bone, which would be capable of transmitting vibrations from distant sources.

Gottschaldt, Fruhstorfer, Schmidt and Kraft (1982) reported that some Herbst units in the goose beak are cold-sensitive. 57.5% of their sample of Herbst units were exited by cooling the receptive field with an ice-cold thermode. Two types of cold-responsive Herbst units were distinguished. The first type responded to the ice-cold thermode with a continuous discharge, with a frequency of up to 80 ips. Using a controlled thermal stimulator, they estimated that the maximum response of the two units reported occurred at 0°C and 15-25°C respectively. The second type of cold-sensitive Herbst unit responded to the ice-cold thermode with a transient discharge of up to 60 ips. The magnitude of this response was reported by Gottschaldt et al (1982) to be dependent upon the amplitude of the temperature drop.

No reports could be found of Herbst units being excited by heating. Conduction velocities of the afferent fibres

innervating the Herbst units were measured by Gottschaldt (1974a), Dorward and McIntyre (1971) and Leitner et al (1973). The largest range reported was from 24-60 m/sec (Dorward and McIntyre, 1971), those reported by Gottschaldt (1974a) and Leitner et al (1973) falling within this range. It can therefore be concluded that the Herbst units are innervated by myelinated fibres in the A-beta range of conduction velocities, with some overlap into A-delta.

All the authors who have described Herbst units have explicitly assumed that a morphologically distinct structure, the Herbst corpuscle, was responsible for the physiologically characterised afferent response.

Direct evidence supporting this structural/functional correlation was provided by Dorward (1970). She surgically exposed individual Herbst corpuscles in the duck wing, and recorded from their afferent axons whilst directly stimulating the corpuscles. The exposed corpuscle responded to a vibrating mechanical probe. No quantitative data were reported by Dorward (1970) except that one corpuscle responded with a 1:1 response up to 390 ips. No other physiologically identified, intact, receptor type in the duck wing followed a vibratory stimulus above 150-200 Hz, if at all, apart from the Herbs units. This direct evidence extends Skoglund's earlier (1960) observations on the responses of surgically exposed Herbst

corpuscles. Whilst the evidence is not extensive it reinforces the view, based on indirect evidence of structural similarity to the mammalian Pacinian corpuscle, that the Herbst corpuscle is the structure responsible for the Herbst unit afferent response.

(ii) The Herbst Corpuscle

Structure

The Herbst corpuscle was first described in the nineteenth century (Herbst, 1848). Many anatomical descriptions of Herbst corpuscles have subsequently been published. The descriptions vary only in minor detail. The following account is based on that of Gottschaldt et al (1982) of the Herbst corpuscle of the goose beak. Variations on this description are presented subsequently.

The Herbst corpuscle is an ovoid body, about 150 µm long and 80µm wide (fig.1:5). It consists of a capsule, an outer zone and an inner core. The capsule is an extension of the perineurium and consists of 5 - 8 layers of perineurial endothelial cells. The outer zone is a fluid-filled enlargement of the endoneural space. It contains a network of collagenous fibres, which are coiled around the inner core. The fibres merge with the basement membrane of the capsule and of the inner core.

The inner core is formed by a double row of 8 - 12 modified Schwann cells, satellite cells, which form two symmetrical lines one on either side of the nerve terminal. The nuclei of these cells project out from the inner core to the outer zone and the cells have cytoplasmic extensions (lamellae). These

extend around the receptor axon towards the opposite satellite cells. The lamellae interdigitate in an irregular fashion with the lamellae arising from the neighbouring satellite cells on the same and opposite side of the receptor axon. Between 60-80 of these lamellae surround the receptor axon. The lamellae are 50 nm thick, with an interlamellar space of 15-20 nm. There are many desmosome-like contact zones between adjacent lamellae.

The receptor axon enters the capsule from one pole. The parent myelinated axon loses its myelin sheath at the beginning of the inner core. The receptor axon itself is ovoid, 1.5 x 3.5 µm, and terminates in a bulb-shaped enlargement, approx. 10µm in diameter. The axoplasm contains neurofilaments, neurotubules, mitochondria, and other cytosomes, and dense-core vesicles

A conspicuous feature of the receptor axon is the presence of finger-like processes of the axon membrane. These processes, 0.5 - 3µm long and 100 - 250 nm diameter, project between the lamellae of the inner core. The axoplasm at the base of these processes lacks mitochondria and cytosomes, and is filled with clear-core vesicles, 30-90 nm diameter. Within each process are longitudinally orientated neurofilaments which project into the receptor axon.

There are many desmosome-like contacts between the receptor

axon and the satellite cell lamellae. These contacts are numerous around the base of the axon processes, but are not present on the axon processes themselves.

No unmyelinated nerve fibres were observed by Gottschaldt et al (1982) in the lamellar system of the inner core.

Structural Variations

Variations in the size and shape of Herbst corpuscles within and between species have been reported. In the duck beak they are reported as ovoid, 150 x 100 µm in size (Quilliam, 1966; Berkhoudt 1980) occasionally up to 190 x 120 µm (Saxod, 1978). In the chicken beak, ovoid 100 x 50 µm (Andersen and Nafstad, 1968) and elongated 25 x 12 µm, up to 300 x 100 µm (Saxod, 1967) variations have been described. In the pigeon articular capsule small, 80 x 20 µm up to 300 x 50 µm, and large, 300 x 60 µm up to 600 x 120 µm, variations were described by Halata and Munger (1980).

Biaxial Herbst corpuscles, i.e. a branching receptor axon forming two inner cores, have been described in the chicken (Saxod, 1967; Winkelmann and Myers, 1961) and the pigeon (Malinovsky and Zemanek, 1969; Halata and Munger, 1980). Bifurcated corpuscles have also been reported in the pigeon (Malinovsky and Zemanek, 1969, 1971).

Variations in the internal structure of the Herbst corpuscles have been reported. The width of the capsule has been reported to vary from 3µm (Berkhoudt, 1980) to 8µm. (Quilliam, 1966) in the duck. The number of lamellae in the outer core has also been reported to vary eg. in the duck Quilliam (1966) described a range of 5-20 lamellae, Saxod (1978) also in the duck reports 15-20 lamellae. The fluid within the capsule contains an acid mucopolysaccharide (Winkelmann and Myers, 1961; Berkhoudt, 1980).

The number of satellite cells in the inner core is variable. In the duck there are 8-12 cells according to Berkhoudt (1980), 20 cells according to Saxod (1978). In the chicken Saxod (1978) reports 50 satellite cells. The regular symmetrical arrangement of the satellite cell nuclei was not observed in the pigeon by Malinkovsky and Pac (1980). Here the satellite cell nuclei were arranged irregularly. This irregular arrangement was also noted by Malinovsky and Zemanek (1971) in the Herbst corpuscles of the chicken glabrous skin. Interestingly they noted a difference in the Herbst corpuscle in different types of skin, the corpuscle in the chicken feathered skin having a regular arrangement of the satellite cell nuclei.

The number of inner core hemilamellae is also reported to vary, e.g. 20-50 (duck, Saxod, 1978), 60-80 (duck, Quilliam, 1966)

50 (chicken, Saxod, 1978) and from 2-60 in the joint capsule of the pigeon (Halata and Munger, 1980).

The dimensions of the receptor axon have been reported as 2x3 µm. (Quilliam, 1966) and 3x5 µm. (Saxod, 1978) in the duck. Saxod also described two types of vesicles in the axoplasm, a clear type of 40-80 nm diameter and a dense core type of 60-100nm diameter.

A secondary innervation in chicken Herbst corpuscles has been observed by Nafstad and Andersen (1970). Unmyelinated axons, which ran both circularly and longitudinally between the inner core lamellae, were seen. Nafstad and Andersen (1970) also described dilatations on these unmyelinated axons, and desmosome-like junctions between the dilatations and the core lamellae.

Occurrence

Herbst corpuscles were first described in 1848, located on the inner side of the base of the metacarpal bone in chicken and pigeon (Herbst, 1848). Subsequent descriptions have shown that they are widely distributed in the avian body. They have been described in the feathered and glabrous skin and mucous membranes in many regions in birds, e.g. the foot and leg (Lucas and Stettenheim, 1972; Burns et al, 1970; Winkelmann and Myers, 1961; Quilliam and Armstrong, 1963; Saxod, 1967,1978),

the wing (Saxod, 1978; Dorward, 1970), the eyelid (Winkelmann and Myers, 1961; Malinovsky, 1966), the ear skin (Malinovsky and Zemanek, 1971), the wattles (Malinovsky and Zemanek, 1971), the comb (Malinovsky, 1966; Malinovsky and Zemanek, 1971) and the tongue (Saxod 1967, 1978. Berkhoudt, 1980). They are also found in joint capsules (Polacek, Sklenska and Malinovsky, 1966; Malinovsky and Zemanek, 1970. Halata and Munger, 1980) and in muscles (Cobb and Bennet, 1970). In the feathered skin, their association with the feather follicles has been noted by Saxod (1967,1978) and Winkelman and Myers (1961). The Herbst corpuscles were found around the base of the follicles, usually one or two in relation to one follicle, with the long axis of the corpuscle at 90° to the feather axis. Saxod (1978) also mentions finding Herbst corpuscles beneath the erector muscles of the feathers.

Herbst corpuscles have been described in the beaks of the four avian species studied physiologically. They were reported in tne chicken beak by Calhoun (1954); Winkelmann and Myers (1961); Malinovsky (1966); Andersen and Nafstad (1968); Nafstad and Andersen (1970); Wight, Siller and Mackenzie (1970), Malinovsky and Zemanek (1971) and Lucas and Stettenheim (1972). Lucas and Stettenheim (1972) stated that the Herbst corpuscles were "more abundant in the anterior half of the upper beak where they are concentrated near the cutting edge". This finding is corroborated by Malinovsky and Zemanek (1971) who

found Herbst corpuscles in the beak tip and in the palate and floor of the mouth. The location of Herbst corpuscles in the tip of the beak is disputed by Wight et al (1970) who found that the most anteriorly situated Herbst corpuscles were located 1/4 to 1/3 of the distance from the tip to the anterior edge of the nares.

Wight et al (1970) report that Herbst corpuscles were present in both the dorsal i.e. rhinothecal and ventral i.e. hard palate, sides of the upper beak. The corpuscles beneath the rhinotheca were situated close to the premaxillary bones. The corpuscles in the hard palate were located underneath the stratum cylindricum of the epidermis, 110µm from the stratum cylindricum according to Nafstad and Andersen (1970). Wight et al (1970) report a difference in size of the corpuscles according to their location. Corpuscles in the rhinothecal region were approximately four times the size of the ones in the palate (mean cross sectional area of the corpuscles was 444.2µm^2 in the rhinotheca, and 91.7µm^2 in the hard palate).

Herbst corpuscles have been reported in the beak skin of the pigeon (Malinovsky and Zemanek, 1969; Malinovsky and Pac, 1980), the goose (Gottschaldt and Lausmann, 1974b; Gottschaldt et al , 1982) and the duck (Quilliam, 1966; Saxod, 1967,1978;

Berkhoudt, 1980). In the duck (Quilliam, 1966; Berkhoudt, 1980) and goose (Gottschaldt and Lausmann, 1974b) Herbst corpuscles were also found in bony lacunae under the horny beak tip (see fig.1:8). In the duck beak skin, the Herbst corpuscles were located approximately 100µm from the epidermis according to Quilliam (1966), and 50-100µm from the epidermis according to Berkhoudt (1980). Gottschaldt and Lausmann (1974b) reported a difference in dermal location between two different species of goose. In the domestic goose (Anser anser) the corpuscles were located 70-310µm below the epidermis, whilst in the white-fronted goose (Anser albifrons) they were found 10-120µm below the epidermis.

The density of Herbst corpuscles in the beak skin of the duck was estimated by Berkhoudt (1980). He found that the density increased towards the side and also towards the tip in the upper and lower beak, for example 5 corpuscles/mm posteriorly, 50/mm at the tip and 100-120/mm in the ridge adjoining the horny maxillary nail. Gottschaldt and Lausmann (1974b) reported that the numbers of Herbst corpuscles also increased towards the anterior part of the beak of the goose. By extrapolation from the numbers counted in a longitudinal strip of beak skin, they estimated that approximately 7,000 Herbst corpuscles were present in the beak skin of the goose.

Comparison of Herbst and Pacinian corpuscles

The Herbst corpuscle has been considered as the avian homologue of the mammalian Pacinian corpuscle. It has been established that the Pacinian corpuscle acts as a rapidly-adapting, vibration-sensitive mechanoreceptor. By analogy with this structural/functional correlation it has been assumed by all the authors who have recorded from avian Herbst units that the receptor responsible was the Herbst corpuscle. For comparison with the Herbst corpuscle, a short description of the anatomy and physiology of the Pacinian corpuscle is included here.

The structure of the Pacinian corpuscle has been described many times. The following description is based on that of Quilliam (1966). The corpuscle is ellipsoid with a mean length of 1mm. and mean diameter of 0.7mm. It consists of a capsule comprised of 7-8 lamellae, each 2 - 3 μm thick, an outer core of 30-60 concentric lamellae in a fluid-filled space, and an inner core of 50-80 Schwann cell hemilamellae surrounding an unmyelinated nerve terminal which has a terminal expansion. Like the Herbst corpuscle, the nerve terminal possesses axon processes containing microfilaments and which penetrate the hemilamellae of the inner core (Andres and Von During, 1973). The electrophysiological characteristics of the Pacinian corpuscle are comparable to those of the Herbst corpuscle. It is rapidly adapting and vibration sensitive. It follows a sinusoidal mechanical stimulus over the frequency range of 60 - 900 Hz

(Hunt, 1974). It is established that in the Pacinian corpuscle, the outer and inner core lamellae together with the fluid in the outer core act as a high-pass mechanical filter, allowing the high-frequency components of a mechanical displacement to reach the nerve terminal but filtering out any steady or low frequency components (Loewenstein, 1971; Loewenstein and Skalak, 1966). It has been proposed that the lamellae of the Herbst corpuscle act in a similar fashion (Gottschaldt et al, 1982). It has been suggested that the axon processes of the Pacinian corpuscle (Spencer and Schaumburg, 1978) and of mechanoreceptor axons in general (Iggo and Muir 1969; Iggo and Andres 1982) are the site of mechanoelectric transduction. A similar suggestion was proposed by Gottschaldt et al (1982) for the Herbst corpuscle, and they have further suggested that a mechanical deformation of the inner core lamellae would result in a deflection or displacement of the axon processes relative to the surrounding receptor membrane. This displacement of the axon processes would occur because these processes can move freely between the lamellae, whilst the rest of the axon membrane is fixed to the lamellae by the desmosomes. Such displacements could activate mechanically-controlled membrane channels leading to an ionic current flow and thus to a receptor potential.

Function of Herbst Corpuscles

The biological function of the Herbst corpuscles would seem to be that of a vibration detector, which would be of great behavioural significance to the bird. For example, in the leg, they could signal weak vibrations of the ground or perch and thus give a warning of the approach of predators (Schwartzkopff, 1948,1949; Dorward and McIntyre, 1971; McIntyre, 1975). In the wing, the Herbst corpuscles associated with feather follicles would be expected to respond to movements of the flight feathers and could therefore provide useful information for flight regulation. McIntyre (1975) has proposed that they could function as stall indicators. In the beak they could supply useful tactile information during preening, exploring, pecking, straining and mandibulation of food particles.

(iii) Grandry Units

Grandry units are specifically velocity-sensitive mechanoreceptors. Detailed information on this physiologically identified receptor type has been obtained in the goose beak by Gottschaldt (1974a,b) and Gottschaldt and Lausmann (1974a), and in the duck beak by Leitner and Roumy (1974a). The most extensive and quantitative study is that of Gottschaldt (1974a). Like the Herbst units, the Grandry units responded only to a changing mechanical stimulus and did not discharge during a maintained skin indentation. Grandry units were distinguished from Herbst units by their high sensitivity to velocity and low sensitivity to vibration. The Grandry units could not follow mechanical vibrations at frequencies greater tha n 200 Hz, the majority only following vibrations up to 100-150 Hz (Gottschaldt, 1974a). A consistent relationship between the discharge frequency and the stimulus velocity was reported by Gottschaldt (1974, a,b), Gottschaldt and Lausmann (1974a) and Leitner and Roumy (1974a). This relationship was described by a power function (Gottschaldt, 1974a,b; Gottschaldt and Lausmann, 1974a). The minimum velocity threshold, ie. critical slope, was 0.25 mm/sec - >5mm/sec (Gottschaldt, 1974a). There was no amplitude sensitivity.

The minimum mechanical force required to excite the units, measured with von Frey hairs, ranged from <5mg to 6.5g

(Gottschaldt,1974a). The comparable force threshold for the Grandry units reported by Leitner and Roumy (1974a) in the duck beak was 0.01g -25g. on the dorsal surface, 25g on the ventral surface, and 1-25g on the tongue.

Grandry units were not excited by cooling or warming (Gottschaldt et al, 1982).

Receptive fields were described by Gottschaldt (1974a) for the Grandry units in the goose beak. They ranged from <1mm up to 12mm in diameter. The larger receptive fields were reported to incorporate two or more minimal threshold points. Gottschaldt (1974a) suggested that this could be the result of the innervation of several receptors by a single afferent fibre. He also reported that the majority of the Grandry units had their receptive fields in the anterior one third of the beak skin and that the receptive fields of different units overlapped, often a large receptive field enclosing a smaller one.

Conduction velocities of the afferent fibres innervating the goose Grandry units ranged from 27.5 to 64.6 m/sec, mean of 42.3 m/sec (Gottschaldt 1974a). Like the Herbst units they are innervated by myelinated fibres in A-beta conduction velocity range.

Gottschaldt (1974a,b) has provided evidence that the responses described for the Grandry unit originated from a morphologically distinct receptor structure, the Grandry corpuscle. After physiologically characterizing a unit and mapping out the receptive field, he punctured the skin at the lowest threshold point, or points, with an ink-filled pipette. Subsequent histological examination of this tissue revealed a Grandry corpuscle beside each penetration tract. No Herbst corpuscle was found within a radius of 250µm, making it unlikely that Herbst corpuscles were responsible for the physiological response. Another strategy employed by Gottschaldt (1974 a,b) was to prick two insect needles into the skin on either side of a low threshold point, then to replace each needle with a thin vibrissa of a cat. Subsequent histological investigation revealed a Grandry corpuscle between these two vibrissae. This work is of course open to the criticism that given the large number of Grandry corpuscles present in the goose beak skin (approx 70,000 according to Gottschaldt and Lausmann (1974b)), it is possible that one would encounter a Grandry corpuscle close to any penetration tract without it necessarily being responsible for the physiologically - characterised afferent unit. Unequivocal evidence for the Grandry unit/Grandry corpuscle correlation might be provided by direct manipulation of the surgically exposed receptor, but this is not feasible given its size and location (see below), or perhaps by intraaxonal recording in

the peripheral nerve and subsequent retrograde HRP transport to directly visualize the structure responsible for the discharge.

(iv) The Grandry Corpuscle

Structure

Grandry corpuscles were first reported in the nineteenth century (Grandry, 1869). A comprehensive description of the structure of the Grandry corpuscle has been provided by Halata (1971), based on his studies of corpuscles in the beak of the goose and swan. The following is based on his description.

The Grandry corpuscle (fig.1:6.) is ovoid in shape, 30-120 µm. in diameter. It consists of a capsule, Schwann cells, Grandry cells and the terminal disc of the afferent nerve fibre. The capsule is incompletely formed and consists of fibroblasts and collagen cells. The Schwann cells enclose the Grandry cells and they possess lamellae which fit between the lamellae of neighbouring Schwann cells. The surface of the Schwann cells is covered with a basement membrane.

The two or sometimes more, Grandry cells are hemispherical, 20-80 µm diameter. Their flat surfaces surround the disc shaped nerve ending. The cytoplasm of the Grandry cell contains glycogen granules, dense core membrane-bound vesicles (70-120 nm diameter) and microfilaments. There are many finger-like processes which project from the hemispherical surface of the Grandry cells. These processes interdigitate with folds on the inner surface of the Schwann cells.

Microfilaments extend into these processes of the Grandry cells.

The corpuscle is innervated by a single myelinated nerve fibre which loses its myelin sheath before entering the capsule. The Schwann cell sheath is lost as it penetrates between the two Grandry cells, and the nerve ends as a disc-shaped terminal plate 20-70 µm diameter and 2-3 µm thick. The axoplasm contains many mitochondria, neurotubles and small vesicles.

The size of the corpuscles has been variously reported in the duck beak as being 30-80µm (Quilliam, 1966), 60µm (Munger, 1971), and 30-70 µm (Berkhoudt, 1980), and 20 - 60µm. in the goose beak (Gottschaldt and Lausmann, 1974b). The number of Grandry cells in the capsule is commonly two, but departures from this norm have been observed. Thus Halata (1971) described 2-4 cells in the goose and swan beak. In the duck beak, Quilliam (1966) reports 2-3 cells, Saxod (1978) up to 7 cells, and Berkhoudt (1980) 2-5 cells. There may be species differences, e.g. in the domestic goose (Anser anser), 47.2% of Grandry corpuscles possessed 2 Grandry cells, 17.6% had 4 cells, and 8.4% >4 cells, whereas in the white-fronted goose (Anser albifrons) 32.6% of corpuscles contained 2 cells, 32.1% contained 4 cells and 14.6% >4 cells (Gottschaldt and Lausmann, 1974b). The largest number of cells in a Grandry corpuscle was that recorded by Gottschaldt and Lausmann (1974b) who found up

to 12 cells in the corpuscle of the goose beak.

In all Grandry corpuscles, neighbouring Grandry cells have a nerve terminal disc sandwiched between them, the discs originating from the parent afferent fibre innervating the corpuscle (fig.1:6).

It has been reported (Saxod 1978) that a single nerve fibre can branch to supply more than one corpuscle. It was also reported by Saxod (1978) that fine unmyelinated nerve fibres were frequently observed around the corpuscles.

Saxod (1978) has described two sorts of vesicles in the nerve disc axoplasm, clear vesicles 40-80 nm diameter and dense core vesicles 60 - 100 nm diameter.

Junctions were noted between the nerve terminal and the Grandry cells in the duck (Andres, 1974; Saxod, 1978). These junctions were usually asymmetric and in some instances the dense-core vesicles of the Grandry cells were seen in close apposition to a membrane thickening. Gottschaldt and Kraft (1978) only rarely observed such junctions and found that the dense-core vesicles were usually concentrated away from the nerve terminals. However, they did not indicate from which species they obtained their material. If it was the goose, the species usually employed by Gottschaldt, then the discrepancy

could reflect a species specific difference. Gottschaldt and Kraft (1978) also report that the axon terminals possess tongue shaped processes containing clear vesicles and neurofilaments. These processes project between a system of microvilli projecting from the Grandry cells. The microvilli contain microfilaments extending into a microfilament network inside the Grandry cells.

Occurrence

Grandry corpuscles are found in the beak and tongue of the duck (Quilliam, 1966, Munger 1971, Saxod, 1973, 1978, Berkhoudt, 1980) and in the beak of the goose (Halata, 1971; Gottschaldt and Lausmann, 1974a,b) and various other aquatic birds. They are not found in the chicken or pigeon (Saxod, 1973, 1978).

The Grandry corpuscles are located in the dermis. In the duck they are found 50-100 µm (mean 75 µm) below the epidermis (Berkhoudt, 1980), in the domestic goose 20 - 150 µm (mean 76 µm) and in the white-fronted goose 1-80 µm (mean 31 µm) below the epidermis (Gottschaldt and Lausmann, 1974). They are found intermingled with Herbst corpuscles, and groups of Grandry corpuscles occur together. The Grandry corpuscles are oriented so that the nerve terminal is parallel to the skin surface (Munger, 1971; Gottschaldt and Lausmann, 1974; Berkhoudt, 1980).

The corpuscles are most numerous towards the sides and distal end of the beak in duck and goose (Quilliam, 1966; Gottschaldt and Lausmann, 1974; Berkhoudt, 1980). For example, Berkhoudt (1980) described densities of 5 corpuscles/mm^2 in the posterior part of the beak and 10-15/mm^2 at the tip. Gottschaldt and Lausmann (1974a,b) estimated approximately 68,000 Grandry corpuscles in the whole of the beak skin in the goose.

Grandry corpuscles were not found in the bony lacunae in the tip of the beak of the duck or goose (Quilliam, 1966; Gottschaldt and Lausmann, 1974b; Berkhoudt, 1980). Whilst Berkhoudt (1980) did not find any Grandry corpuscles under the horny nail in the duck, Quilliam (1966) and Gottschaldt and Lausmann (1974b) found a few in this location in duck and goose respectively. The Grandry corpuscles observed by Gottschaldt and Lausmann (1974b) under the horny nail were smaller in diameter (mean 30µm) than the corpuscles in the beak skin. The horny nail corpuscles rarely had >4 cells per corpuscle, and they were situated deeper in the epidermis, 70-220 µm, mean 146 µm, than the corpuscles in the beak skin.

Grandry corpuscles are not present in the beaks of the chicken and pigeon. However, the Merkel corpuscle, which is morphologically very similar, although smaller in size, has been observed in these species.

(v) The Merkel Corpuscle

Structure

The Merkel corpuscle structure has been described by Andersen and Nafstad (1968), Saxod (1978) and Ide and Munger (1978). The following description is based on that of Andersen and Nafstad (1968).

The Merkel corpuscle is composed of lamellar cells, Merkel cells and disc-shaped nerve terminals. The lamellae are Schwann cells with cytoplasmic processes. Small groups of collagen fibres are found in the interlamellar spaces.

The Merkel cells are discoid in shape, 10 x 3 µm. Around the equator of the Merkel cells, finger-like processes, 1 - 2 µm long, project into the surrounding connective tissue. The Merkel cell cytoplasm contains bundles of microfilaments which run into these processes. The cytoplasm alsocontains membrane-bound dense core vesicles, diameter approximately 130 nm, found singly and in groups.

The nerve terminals are disc-shaped, 0.5 - 2µm thick. The axoplasm contains many mitochondria and vesicles, 60 nm diameter. Synaptic junctions between the nerve terminal and Merkel cells have been described by both Andersen and Nafstad

(1968) and Saxod (1978), who examined the chicken palate. The junctions consisted of a thickened membrane on the nerve ending side, with a group of dense-core vesicles on the Merkel cell side. These junctions were not seen by Ide and Munger (1978) in Merkel corpuscles from the chicken toe skin.

Occurrence

Merkel corpuscles have been observed in the hard palate of the chicken (Andersen and Nafstad, 1968, Wight et al, 1970; Nafstad 1971, Saxod, 1978) and pigeon (Merkel, 1875), and in the tongue (Merkel, 1875) and toe skin (Ide and Munger, 1978) of the chicken. All the authors reported a dermal location for the corpuscles, with the exception of Wight et al (1970) who reported an epidermal location.

The orientation of the Merkel corpuscles is such that the long axis of the Merkel cells is parallel to the epidermis (Andersen and Nafstad, 1968; Saxod, 1978; Ide and Munger, 1978). Andersen and Nafstad (1968) and Saxod (1978) report that in the palate they are found in groups of two or more in the dermal papillae.

Comparison of Grandry and Merkel corpuscles

The Grandry and Merkel corpuscles are obviously very similar cytologically. The Grandry and Merkel cells both have dense core vesicles and microfilaments, and the same relationship to the nerve ending. The distinction between the two sorts of corpuscles has been made on the grounds of distribution and size (Saxod, 1978). Saxod (1978) considers that Grandry and Merkel corpuscles are two varieties of the same receptor. There is some dipute over terminology. Ide and Munger (1978) are of the opinion that the term Grandry corpuscle should be used to describe both the Grandry and Merkel corpuscles, on the grounds of morphological, physiological and embryological similarity. With regard to the physiological characteristics, the evidence obtained by Gottschaldt (1974) strongly suggests that the Grandry corpuscles function as rapidly adapting mechanoreceptors. No direct evidence exists for a comparable function for the Merkel corpuscles. However, evidence for physiologically identified rapidly adapting mechanoreceptors was obtained by Roumy and Leitner (1973) in the chicken beak and it is possible that the "vibration-insensitive" rapidly adapting units had their origin in the Merkel corpuscles. From the embryological viewpoint, the Grandry and Merkel cells are thought to have a common origin, in the neural crest (Saxod, 1978; Ide and Munger, 1978).

Mammalian Merkel Cell

A structure bearing close resemblance to the general features of the avian Grandry and Merkel corpuscles has been identified in mammals, the Merkel cell-neurite complex (Merkel, 1875; Iggo and Muir, 1969). The mammalian Merkel cell-neurite complex, however, has an epidermal location and different functional properties. It has been unequivocally identified as a slowly adapting mechanoreceptor, known as the slowly adapting type I unit (SA1) (Tapper, 1965; Iggo, 1966a; Iggo and Muir, 1969; Munger, Pubols and Pubols 1971; Gottschaldt, Iggo and Young, 1973). The prediction that the avian Grandry corpuscles, by analogy with the structural similarity to the mammalian Merkel cell receptors, would similarly function as slowly adapting mechanoreceptors (Iggo, 1966b; Munger, 1971; Andres and von During, 1973) has not been borne out by the results of Gottschaldt (1974a,b). Furthermore the Merkel cell receptors in the salamander skin have been identified as rapidly adapting mechanoreceptors by Parducz, Leslie, Cooper, Turner and Diamond (1977). Gottschaldt and Kraft (1978) proposed that, in view of the fact that morphologically similar specialized cells, i.e. Merkel cells and Grandry cells, accompany the nerve terminals of mechanoreceptors with different functional properties, the same "terminal cell receptor" should be applied to all these receptor types.

Two opposing schools of thought exist regarding the function of

the mammalian and avian terminal cells. One school suggests that the terminal cells are secondary sensory cells, deformation of the cells inducing a generator potential which would release a chemical transmitter from the dense-core granules at the contact zone between the terminal cells and the nerve endings (Iggo and Muir, 1969; Horch, Burgess and Whitehorn, 1974; Hartschuh and Weihe, 1980; Cooksey, Findlater and Iggo, 1984). The other school of thought suggests that the terminal cells are not secondary sensory cells, but instead function as mechanical filters modulating stimuli and transmitting them to the nerve terminal where the transduction process takes place (Gottschaldt and Kraft, 1978; Gottschald and Vahle-Hinz, 1981, 1982). Evidence exists to support both schools of thought and the problem is, as yet, unresolved.

Function of Grandry corpuscles

The biological function of the rapidly adapting mechanoreceptor identified with the Grandry corpuscle in ducks and geese, and tentatively associated with the Merkel corpuscle in chicken and pigeon, could be to provide information about contact and movement during a variety of behavioural actions using the beak, e.g. preening, pecking, straining and manipulation of food particles. No evidence is available concerning the physiological characteristics of the Merkel corpuscles in the toe skin of the chicken.

(vi) Other observations on avian RA mechanoreceptors.

The evidence for rapidly adapting mechanoreceptors in the pigeon beak is not extensive. Zeigler, Miller and Levine (1975), recording extracellularly from the trigeminal ganglion, found units which were responsive to mechanical stimulation, but no differentiation into rapidly and slowly adapting units was made. In the wing skin of the pigeon Necker (1983) found evidence of two types of RA mechanoreceptors but no substantial data were provided. More data are required to clarify the situation.

(b) Slowly adapting mechanoreceptors

Slowly adapting mechanoreceptors have been reported in the beak of the goose (Gottschaldt, 1974a), the duck (Necker, 1974a) and the pigeon (Necker, 1972, 1973,1974a,b, 1983), and in the wing skin of the duck (Dorward, 1970) and the pigeon (Necker, 1979; Necker and Reiner, 1980). By comparison with the rapidly adapting mechanoreceptors little detailed quantitative information is available. Gottschaldt (1974a) has , however provided a detailed description of slowly adapting mechanoreceptors, in the beak of the goose. A large proportion (2/3) of his sample of 17 slowly adapting mechanoreceptor units had a spontaneous discharge with either a regular or an irregular interspike interval. A static maintained indentation of the receptive field of all of the SA units produced a continuous discharge. Using a ramp and hold mechanical stimulus, Gottschaldt (1974a) identified two components of the afferent discharge. These were an initial dynamic response, in which the discharge frequency increased with the velocity of the ramp, followed by a static response which lasted for the duration of the stimulus, in which the discharge frequency increased with the indentation amplitude. Thresholds and stimulus-response relationships for these two response components were not quantified by Gottschaldt (1974a). He did, however, illustrate one unit responding to an indentation of 50um amplitude. The release of this indentation produced a "silent period", i.e. no spontaneous discharge after the

cessation of the stimulus. The duration of this "silent period" increased with the ampitude of the preceding displacement (Gottschaldt, 1974a).

Necker (1972,1973,1974,a,b) gives a qualitative description of two types of slowly adapting mechanoreceptor discharge in the pigeon beak. The first type had a spontaneous regular discharge. During maintained mechanical indentation there was a sustained discharge with a regular or irregular discharge pattern. The second type had no spontaneous discharge and responded to a maintained mechanical indentation with a sustained irregular discharge. The duck beak was reported to have the second type of slowly adapting mechanoreceptor (Necker, 1974a).

In the pigeon wing, Necker, 1979) and Necker and Reiner (1980) report slowly adapting mechanoreceptors of the second type described above. Dorward (1970), in the duck wing, reported units which she described as "touch receptors". These units had no spontaneous discharge, and responded with a sustained discharge to a maintained deformation of the receptive field with a glass stylus. No quantitative data were presented.

The only description of receptive fields of slowly adapting mechanoreceptors is that of Gottschaldt (1974a). The receptive fields were present in the palate of the anterior part of the

beak, the horny premaxillary plate at the tip of the bill, and the bill tip organ (see below for details of this structure). The receptive fields were measured on the premaxillary plate, and were 1-5um in area. In some of the units found in the premaxillary plate, a directional sensitivity was observed. Bending the plate in one direction caused an increase in the discharge frequency, whereas bending it in the opposite direction caused a decrease in discharge frequency (Gottschaldt, 1974a).

Temperature Sensitivity of the SA mechanoreceptors

Gottschaldt (1974a) and Necker and Reiner (1980) both reported that some of the slowly adapting mechanoreceptors were temperature sensititve. Necker and Reiner (1980) found that 3 out of 15 of their units in the pigeon wing, not spontaneously active, were excited by radiant cooling (stimulus and response parameters not quantified). Gottschaldt (1974a) observed that the spontaneous activity of "most of" the goose beak slowly adapting mechanoreceptors was increased on cooling the receptive field with ethyl chloride, and decreased after warming with water. Detailed descriptions of the stimulus and response parameters were not given.

This thermal sensitivity was investigated more closely by Gottschaldt et al (1982). In their sample of 29 slowly adapting mechanoreceptors with receptive fields in the horny

tip of the beak, 18 (62%) were temperature sensitive. The response to both constant temperature, (static temperature response), and to changing temperature, (dynamic temperature response) was examined, using measured and controlled stimulus temperatures of 5- 45°C. The static temperature response varied from unit to unit, the units exhibiting maximum discharges at temperatures varying from 10 to 30°C. The temperature ranges over which the units were active also varied, from as low as 5 °C to as high as 45°C. A rapid temperature change always produced an increased discharge frequency during cooling, and a decreased frequency during warming. Gottschald et al (1982) noted that 6 of the slowly adapting mechanoreceptors displayed a bursting discharge pattern at lower static temperatures. In their illustrated example, the unit discharged with a regular interspike interval at 40°C. At 20°C, bursting appeared. The number of impulses in a burst, the interburst interval, and the intraburst interval all increased as the temperature was changed from 20°C down to 4°C.

The effect of simultaneous thermal and mechanical stimulation on the discharge of the slowly adapting mechanoreceptors has been investigated by Necker (1973,1974 a,b,1979, 1983, Necker and Reiner, 1980) and Gottschaldt et al (1982). Necker used a contact thermode to produce a maintained mechanical stimulus (parameters not given) and altered the temperature of the thermode to vary the temperature of the receptive field of the

unit under study. He demonstrated both static and dynamic temperature responses. The static response consisted of an increase in frequency of discharge with increase in temperature over the range 10 °C to 45 °C. The maximum response occurred at different temperatures according to the preparation studied, eg. in the pigeon beak the maximum response occurred at 40 °C (Necker, 1973, 1974a), in the duck beak it occurred at 20-30 °C (Necker, 1974a) and in the pigeon wing it occurred at 36 - 43 °C (Necker, 1979; Necker and Reiner, 1980).

Two types of dynamic temperature response were observed by Necker. The first type was observed in the beak (Necker, 1973, 1974b) and the wing (Necker 1979,1983) of the pigeon. This type of response consisted of an increase in discharge frequency during cooling, and a decrease in discharge frequency during rewarming to the original temperature. The second type of response, observed only in the pigeon beak, was the reverse of the first type, ie. decrease in frequency during cooling and an increase in frequency during rewarming to the original temperature (Necker, 1973, 1974b).

Gottschaldt et al (1982) employed a different stimulation strategy. They used a ramp and hold mechanical stimulus, and investigated the effects of heating and coooling on the response to this stimulus. They reported that cooling the receptive field with ice always increased the discharge

frequency. Warming the receptive field (temperature not specified) abolished the static phase of the mechanical response, leaving the dynamic phase of the mechanical response intact but with the discharge frequency reduced.

The conduction velocities of the afferent fibres innervating slowly adapting mechanoreceptors were measured by Gottschaldt (1974a). They ranged from 41.0 m/sec to 55.5 m/sec, mean 49.8 m/sec. They are therefore myelinated fibres of the A-beta conduction velocity range.

The origin of the slowly adapting mechanoreceptor discharge, in the goose beak at least, was thought by Gottschaldt et al (1982) to be a type of receptor known as the Ruffini ending. Direct evidence is not available to support this contention, but it is supported by the indirect evidence of functional and structural analogy to the mammalian slowly adapting mechanoreceptor which has been identified with the mammalian Ruffini ending. Evidence which, although indirect, tends to add weight to this speculation is that the receptive fields of the slowly adapting mechanoreceptors were located in the only regions where Ruffini endings were found, according to Gottschaldt et al (1982). It must be pointed out, however, that Gottschaldt has published contradictory findings. Gottschaldt et al (1982) reported that the receptive fields and the Ruffini endings were found only in the part of anterior

beak covered by the horny plate, and never in the beak skin or the bill tip organ, but in previous reports he demonstrated both receptive fields (Gottschaldt 1974a) and Ruffini endings (Gottschaldt, Andres and von During, 1976) in the bill tip organ.

The Ruffini Ending

The avian Ruffini ending was described in detail by Gottschaldt et al (1982). The following summary is based on that description.

The Ruffini endings were always found associated with collagenous fibres. They were of two types. The first type was associated with oblique collagenous fibre strands which extended from the surface epithelium to the periosteum of the premaxillary bone. The receptors were located close to the periosteum. The receptor consisted of a collagenous fibre bundle, which surrounded Schwann cell processes and a receptor axon. The Ruffini ending was round or oval in cross-section. The Schwann cell processes enclosed the receptor axon branches. The enclosure was not always complete, and in some places the axon membrane was next to the collagenous microfibrils. The Schwann cell cytoplasm contained many vesicles. The surface membranes of the Schwann cells had desmosome-like contact areas, which appeared to connect the Schwann cell processes with each other or with the surface membrane of the receptor

axon.

The afferent nerve fibre lost its myelin shealth before entering the collagen fibre bundle. It branched into many nerve terminals which wound through the collagenous fibre branches. The nerve terminal axoplasm contained mitochondria, neurofilaments and neurotubules, and many clear vesicles which were found close to the membrane. The nerve terminals possessed finger-like axon processes, 300-600 nm long. These processes penetrated into the Schwann cells or occasionally penetrated through the Schwann cell envelope and came into close contact with the collagen microfibrils. The bases of these processes did not contain mitochondria but did contain many clear core vesicles (50-100 nm diameter) some microfilaments and microtubules.

The second type of Ruffini ending was found associated with collagenous fibre strands which ran, in the middle of the dermis, parallel to the surface and across the long axis of the beak. The feature which distinguished this type of Ruffini ending from the first type was the presence of a single specialized cell. This cell was similar to the specialized cells in avian Grandry and Merkel corpuscles. Gottschaldt et al (1982) designated it, in common with the cells in the Grandry and Merkel corpuscles, a "terminal cell". The terminal cell was located close to the receptor axon's initial branching

point. Several axon branches made contact with the terminal cell, at symmetric, asymmetric and desmosome-like junctions. Symmetric and desmosome-like junctions also existed between the Schwann cell processes the terminal cell, the adjacent Schwann cell processes, and and the nerve-ending. The terminal cells possessed microvilli which projected into the surrounding Schwann cells or penetrated them and came into contact with the surrounding collagenous microfibrils.

The observations of Gottschaldt et al (1976,1982) constitute the only report of Ruffini endings in a cutaneous location in birds. The only other location reported for avian Ruffini endings is in joint capsules of chicken, pigeon, and rook (Polacek et al, 1966, Malinovsky and Zemanek, 1970; Halata and Munger, 1980). These Ruffini endings were of the first type of structure described by Gottschaldt et al (1982) in the goose beak, i.e. with no associated terminal cell.

The avian Ruffini ending is very similar to the mammalian Ruffini ending, found in joint capsules (Polacek,1966) and in the dermis of hairy skin (Chambers, Andres, von During and Iggo, 1972; Biemesderfer, Munger, Binck and Dubner, 1978). In hairy skin it has been identified as being responsible for a slowly adapting mechanoreceptor discharge. The structure of the Ruffini ending has been described by Chambers et al (1972). Briefly, the ending was 0.5-2mm long and spindle shaped. It

was approximately 130µm in diameter at the centre, and approximately 30µm diameter at each end. It was enclosed in a capsule composed of 4-5 layers of perineural cells, inside which was a fluid-filled space surrounding a core of nerve terminals, connective tissue and collagenous fibrils. The receptor was innervated by a myelinated nerve fibre which entered either at one end of the receptor or at the centre. The fibre lost its sheath inside the receptor, at the core. Here, it branched, the branches ramifying through the core to both ends of the receptor. The branches contacted the collagen fibrils, with tubular axonal processes, 0.2-1µm in diameter, containing microfilaments, and granulated vesicles at the base of the processes. At the ends of the receptor, the collagen fibrils formed collagen fibres which entered the connective tissue of the dermis.

This receptor type was identified in a correlative electrophysiological and histological analysis, as the morphological substrate of the physiologically identified slowly adapting type II mechanoreceptor (Chambers et al, 1972). Briefly, this receptor type usually had a spontaneous, regular, discharge, and responded to a maintained mechanical stimulus with a sustained regular discharge. Like the avian units described by Gottschaldt (1974a) they showed a dynamic and static response to the velocity and amplitude of the indentation respectively. They showed thermal sensitivity, the

spontaneous discharge and the mechanically-induced discharge being increased by a fall in temperature and decreased by a rise in temperature. These receptors are innervated by myelinated fibres, with conduction velocities of 20-100 m/sec (Brown and Iggo, 1967).

Gottschaldt et al (1982) have proposed that the axon processes on the nerve terminals of the Ruffini endings are the site of mechanoelectric transduction. According to this scheme, m echanical stimuli applied to the beak tip would be transmitted via the collagenous fibre strand to the axon terminals. A maintained stretch of the collagenous tissue would lead to a sustained deflection of the axon processes.

The functional significance of the specialized terminal cell in the second type of avian Ruffini ending described by Gottschaldt et al (1982) is unclear. It has been suggested (Gottschaldt et al, 1982) that it may protect the nerve endings from mechanical damage, or it may alter the amplitude or time course of mechanical stimuli. Gottschaldt et al (1982) also suggest that the terminal cell could influence the regularity of the afferent discharge. As a result of this, the slowly adapting units with the irregular and regular discharge patterns would correspond to the Ruffini endings with and without terminal cells respectively.

3. The Bill Tip Organ

In most birds, the tip of the beak is formed by hard horn, enabling the beak to be used as a tool or weapon. This structural specialization apparently conflicts with the requirement for tactile sensitivity necessary for feeding and preening. However at the tip of the beak of many species a distinctive aggregation of receptors has been observed., firstly in the parrot by Goujon (1869). These receptors and the tissue in which they are embedded have been termed the "bill tip organ" by Gottschaldt and Lausmann (1974b).

It has been described in some detail by Gottschaldt and Lausmann (1974b) in the goose, Berkhoudt (1976, 1980) in the duck and Gentle and Breward (1985) in the chicken. The structures described are very similar and are discussed below.

The gross morphology of the bill tip organ comprises a number of tubules which run through the horn at the beak tip and which end externally in a number of openings on the inner, i.e. buccal, face of the beak tip. The distal ends of the tubules are covered by a keratin cap. The proximal ends of the tubules are open to the dermis underlying the horn of the beak. In the dermis of the tubules are Herbst and Merkel corpuscles in the chicken, Herbst and Grandry corpuscles (duck and goose) (fig.1:7). Gottschaldt and Lausmann (1974b) observed up to 10 Herbst corpuscles and more than 30 Grandry corpuscles per

tubule. Berkhoudt (1980) observed 3-16 Herbst and 4-18 Grandry corpuscles per tubule in the duck. Both authors agree that the Grandry corpuscles are located more distally, closer to the outer opening of the tubules than the Herbst corpuscles which were loated closer to the base of the tubules. Gottschaldt and Lausmann (1974b) reported that the orientation of the Granry cells in the Grandry corpuscles was with their flat surfaces parallel to the distal ends of the tubules, not to the epidermis. The Herbst corpuscles were orientated at random according to Gottschaldt and Lausmann (1974b), but according to Berkhoudt (1980) they were orientated with the nerve terminal's long axis parallel to the distal end of the tubule.

Gottschaldt et al (1976), in a further investigation, observed that in the goose, each tubule is innervated by 40-100 myelinated fibres, 60-85% of which are greater than 3 µm in diameter. In addition, they also observed several other presumptive receptor structures, "Merkel-type" cells below the epidermis in the distal part of the tubules, Ruffini endings, and epidermal free nerve endings in the tubule tip.

A vascular system has been observed in the tubules with a blood vessel forming a loop in the distal end and numerous arterio -venous anastamoses (Gottschaldt and Lausmann, 1974b; Gottschaldt et al, 1976; Berkhoudt, 1980).

The number of tubules varies between species. In the chicken there are 15-20 tubules in a row in the tip of the lower beak, there are none present in the upper beak tip (Gottschaldt and Lausmann, 1974b; Gentle and Breward, 1985). In the goose there are 100 tubules in 1-3 rows in the maxillary nail and 180 tubules in up to 5 rows in the mandibular nail (Gottschaldt and Lausmann, 1974b). Berkhoudt (1977) found that in the duck, the number of tubules was species-specific. In the mallard, shoveler and tufted duck there were 45, 37 and 29 tubules respectively on the maxillary nail, and 187, 120 and 180 tubules respectively in the mandibular nail. In the maxillary nail they were present in 1 row, in the mandibular nail in 3-4 rows. There are no tubules present in the pigeon beak (Gottschaldt and Lausmann, 1974b).

The bill tip organ, with its accumulation of morphologically-identified receptor types, would be extremely useful to the bird, and could provide sensory information vital to such activities as preening and feeding. Physiological data indicate that rapidly and slowly adapting mechanoreceptors are present in the beak tip, at least in the goose (Gottschaldt 1974a). Gottschaldt and Lausmann (1974b) suggested that the horn between the tubules enhances the mechanical isolation of neighbouring tubules and thus increases the potential for spatial resolution of tactile sensory information. The unusual orientation of the Grandry corpuscles suggests that it is a

vertical compression of the Grandry corpuscles from the free surface that induces an afferent discharge. This vertical compression would occur when an object is grasped by the beak. The bill tip organ could relay information relating to many features of the object e.g. food particles. For instance, information relating to the position, through activation of different papillae and the slowly adapting mechanoreceptors; movement, through activation of the rapidly adapting mechanoreceptors; size, through activation of different numbers of papillae and the distance between the upper and lower bill tip organs, and perhaps temperature. The latter function is highly speculative. It is often assumed that epidermal free nerve endings are the morphological substrate of thermoreceptors, and epidermal free nerve endings have been described in the goose bill tip organ by Gottschaldt et al (1976), but thermoreceptive afferent activity has not, as yet, been described in the bill tip organ.

B. CUTANEOUS THERMORECEPTORS

Slowly adapting mechanoreceptors which display some temperature sensitivity have been referred to as "spurious thermoreceptors" (Iggo, 1969). It has been established in mammals that a separate distinct class of receptors exists which are excited only, or preferentially, by thermal stimuli, thermoreceptors (Hensel, 1973). Evidence for the existence of this type of receptor will now be reviewed, firstly in the chicken and subsequently in other avian species.

1. Chicken thermoreceptors

Evidence relating to the existence of cutaneous thermoreceptors in the chicken is scanty and somewhat inconclusive. Two reports refer to temperature-sensitive receptors but the distinction between temperature-sensitive mechanoreceptors and specific thermoreceptors was not made in either case. Roumy and Leitner (1973) reported 4 units from the beak. These units were spontaneously active, the discharge frequency was increased by a drop in temperature, and it ceased when the temperature was increased. No further stimulus or response parameters are given. The receptive fields for these units were located on the "dorsal and ventral side" of the beak. Without any further details, it is difficult to accept this as unequivocal evidence for specific thermoreceptors. It is possible that the 4 units

recorded were, in fact, slowly adapting thermally-sensitive mechanoreceptors as described in the previous section of this review. As Roumy and Leitner (1973) do not mention testing these units for mechanical sensitivity, this possibility remains.

Cold-sensitive receptors in the tongue of the chicken and pigeon were described by Kitchell et al (1959). These units were spontaneously active. Cooling the tongue from 39 to 36 °C with a contact thermode, produced an abrupt increase in discharge frequency. This response lasted 2 seconds, then the discharge frequency decreased to a level slightly higher than the level at 39 °C. Rewarming the tongue from 36 to 39 °C produced a decrease in discharge frequency to the original level. This is unequivocal evidence for thermal sensitivity, but the possibility exists that these units could have been temperature-sensitive slowly adapting mechanoreceptors, as Kitchell et al (1959) used a thermode in mechanical contact with the tongue for thermal stimulation. More data are required in order to clarify the situation.

2. Thermoreceptors in other avian species

The existence of specific cutaneous thermoreceptors has been established in the pigeon (Necker, 1972,1973,1981,1983; Necker and Reiner 1980) and the duck (Gregory, 1973; Leitner et al 1973; Leitner and Roumy, 1974b). The common electrophysiological characteristics described for these thermoreceptors can be summarized in general terms as follows. The thermoreceptors have a spontaneous discharge. They exhibit both static and dynamic responses to temperature changes. At constant skin temperature, the frequency of the discharge is dependent on the temperature value: the static temperature response. During a change in skin temperature, the discharge frequency changes: the dynamic temperature response. The thermoreceptors are insensitive to mechanical stimuli. The latter characteristic distinguishes them from those spontaneously active slowly adapting mechanoreceptors which are also temperature sensitive. Avian thermoreceptors can be divided into two classes, cold receptors and warm receptors, on the basis of the temperature response. During a decrease in skin temperature, there is an increase in discharge frequency in the cold receptors and a decrease in frequency in the warm receptors. At constant skin temperatures, the cold receptors have their maximum discharge at temperatures lower than 30 °C, the warm receptors at temperatures higher than 40 °C.

(a) Cold Receptors

The static temperature response of cold receptors in the beak of the pigeon and duck and also in the tongue of the latter have been described by Necker (1972), Gregory (1973) and Leitner and Roumy, (1974b). An increase in firing rate from 0.5 to 2.5 ips was elicited by temperatures of 26 - 20°C (Necker, 1972). Temperatures below 20°C were not tested by Necker (1972). Leitner and Roumy (1974b) report maximum discharge frequencies at 30-20 °C, but do not give further details. Gregory (1973), employing a more extensive range of stimulus temperatures (2- 40°C), reported an increase in firing frequency from 40°C up to a maximum at 30-15°C, then a decrease in frequency down to 2°C. Maximum discharge frequency was 5 - 8 ips. Dynamic temperature responses were found. The general response to cooling and rewarming the receptive fields of cold receptors was a transient increase in discharge frequency on cooling, followed by adaptation of this discharge frequency to a lower level at maintained lower temperature, and inhibition of discharge on rewarming. For example, Necker (1972) illustrated one unit which discharged at 3 ips at 36°C. A slow cooling from 36°C to 20°C, over 2.5 mins, elicited an increase in discharge frequency to 16 ips. This discharge frequency decreased to 6 ips after 2.5 mins. Rewarming the skin from 20 °C back to 36°C produced a phasic inhibition of the discharge. The time course of this inhibition was not reported.

The dynamic response appears to vary considerably between individual cold receptors. The magnitude of the response appears to increase with the size and rate of the temperature change. Some cold receptors in the pigeon appear to lack any appreciable dynamic response (Necker, 1972; Necker and Reiner, 1980).

(b) Warm Receptors

Avian warm receptors have so far only been recorded in the pigeon beak (Necker 1972,1973). These units were spontaneously active and the discharge frequency was dependent on static temperature, increasing with warming and decreasing with cooling. The example illustrated by Necker (1972) shows an increase in discharge frquency of 0 - 60 ips over the range 20-44 °C. A bursting discharge occurred at lower temperatures (26 °C in his illustrated example).

A dynamic temperature response was reported for the warm receptors by Necker (1972,1973). Three examples were illustrated. Cooling the receptive field from 38 to 24 °C, 40 to 32 °C and 34 to 22 °C respectively produced an inhibition of the discharge frequencies which were 45 ips, 25 ips and 20 ips respectively. The inhibition lasted for the duration of the maintained contant lower temperature. Rewarming back to the

original temperature resulted in an increase in discharge frequency back to the original rate.

(c) Receptive Fields

Receptive fields were described by Gregory (1973) as "small, single areas with diameters of a few mm." Leitner and Roumy (1974b) reported that the receptive fields of their cold receptors were located on the beak tip or in and around the nostrils. All of these receptive fields were "about 12mm" in size (Leitner and Roumy, 1974b). The locations of Necker's (1972,1973) warm and cold receptors were "on the borders of, or inside, the beak" (Necker, 1972,1973). Three warm receptors were located "in the throat" (Necker, 1973). No information was given by Necker about the sizes of the receptive fields.

(d) Morphology of the thermoreceptor

All authors cited have suggested, in common with the generally accepted hypothesis, that the morphological substrate of thermoreceptors consists of epidermal free nerve endings. No evidence exists to support or refute this correlation for birds. Epidermal nerve endings have been histologically reported, as summarized below.

Epidermal nerve endings un associated with any specialized cells have been found in the beak of the chicken (Wight et al, 1970; Malinovsky and Zemanek 1971) and in the beak and tongue of the duck (Saxod, 1978). Malinovsky and Zemanek (1971) found that free nerve endings were present in large numbers at the beak tip. They were also found in the palate and oral mucosa and in the feathered skin at the base of the wattles, ear-flaps, and comb. In the hard palate and rhinotheca, the free nerve endings terminated in the stratum germinativum of the epidermis (Wight et al, 1970).

A detailed description of the free nerve endings of the duck beak was given by Saxod (1978). They were abundant in the upper beak, at the level of the lateral ridges, at the tip of the beak and around the nostrils. Saxod (1978) observed that myelinated nerve fibres penetrated the epidermis principally in the region between the epithelial ridges where the epidermal

layer is thin. The myelin sheath and Schwann cells were lost when the fibres penetrated the epithelial basement membrane. The resulting unmyelinated fibres, up to 1 μm in diameter, had a winding course through the epidermis, and sometimes reached as far as the superficial cornified desquamating layer of cells.

(e) Comparison of avian and mammalian thermoreceptors

The general physiological properties of the mammalian cutaneous thermoreceptors are similar to those of the avian cutaneous thermoreceptors, i.e. an insensitivity to mechanical stimuli, a maintained discharge at constant skin temperatures, the discharge frequency being temperature dependent, and a change in discharge frequency during a change in skin temperature. Both cold and warm receptors have been physiologically identified in mammals. The values of the temperatures at which the maximum static temperature response occurs are comparable with those obtained from avian studies, i.e. 20 to 30°C for cold receptors (Iggo, 1959, 1969; Kenshalo and Duclaux, 1977) and 40 to 47 °C for warm receptors (Hensel, 1973; Duclaux and Kenshalo, 1980). The general features of the dynamic temperature response of the mammalian thermoreceptors are comparable to those of birds, i.e. the cold receptors respond with an increase in discharge frequency to a fall in temperature, and a decrease in discharge frequency to an

increase in temperature, and vice-versa for the warm receptors (Hensel, 1973). A notable difference between mammalian and avian cold receptors is that the latter show lower maximal discharge frequencies for both the static and dynamic response. Maximal discharge frequencies of static and dynamic responses in mammalian cold receptors are around 300 and 20 ips respectively (Hensel, 1973), whereas in birds they are around 8 and 30 ips respectively (Necker, 1972, 1973; Gregory, 1973; Leitner and Roumy, 1974b). Another difference between birds and mammals is that warm receptors in mammals show a bursting discharge at high skin temperatures (Hensel, 1973) whereas they have been observed to exhibit a bursting discharge at low temperatures in the pigeon (Necker, 1972, 1973).

The mammalian thermoreceptors have small receptive fields consisting of one or two adjacent spots, usually less than 1 mm in diameter (Hensel, 1973). The afferent fibres innervating the thermoreceptors have condution velocities under 20 m/sec, i.e. A-delta or C fibres.

The receptor structure responsible for the cold receptor discharge has been identified in the cat facial skin, in a correlative electrophysiological/histological analysis by Hensel et al (1974). Underneath the receptive field of physiologically characterised cold receptors they observed distinctive nerve terminals. Each receptive field was supplied

with a single myelinated fibre, which branched close to the basal epidermal cells. These branches terminated inside the epidermal cells. The axon branches were covered with a Schwann cell layer up to the epidermal basement membrane. The tips of the axon branches penetrated into the basal epidermal cells, the basement membrane of the axon branches being continuous with that of the epidermal cells. The axoplasm in the tips of the branches contained mitochondria, fine filaments and vesicles.

C. CUTANEOUS NOCICEPTORS

The International Association for the Study of Pain (IASP) defines a nociceptor as "a receptor preferentially sensitive to a noxious or potentially noxious stimulus (IASP subcommittee on taxonomy, 1979). A noxious stimulus is defined by IASP as "a tissue damaging stimulus" (IASP subcommittee on taxonomy, 1979). This preferential sensitivity to noxious or potentially noxious stimuli is the criterion by which nociceptors can be distinguished from other cutaneous receptors. Some nociceptors have been described which respond solely to stimuli of tissue damaging intensity (Burgess and Perl, 1973), but the majority have thresholds that are in the innocuous range (see table 1:1). The latter type, however, are excited to maximum response by stimuli of noxious intensity and can encode in their response information about the intensity of stimulation over the continuum from innocuous through to noxious levels (Perl, 1968; Beck, Handwerker and Zimmermann 1974; Torebjork, LaMotte and Robinson, 1984). This differential response to noxious stimulus intensities distinguishes the nociceptors from other cutaneous receptors. The latter transmit information about stimulus intensities in the innocuous range and their maximum response is to stimuli in this range. They may respond to noxious stimulus intensities, but the response is not distinguishable from that elicited by innocuous stimuli (Burgess and Perl, 1973; Price and Dubner, 1977).

The characteristics of mammalian nociceptors have been extensively described. By contrast, there has been little investigation of nociceptors in avian species. A brief review of the well-defined characteristics of mammalian nociceptors is included here as background information against which to assess the data for avian species.

1. Mammalian nociceptors

Cutaneous nociceptors have been extensively characterized in five mammalian species (rat, cat, rabbit, monkey and man). The diversity of receptive field locations and characteristics, stimulus thresholds and afferent fibre conduction velocities of the nociceptors in these species can be appreciated by reference to table 1:1. This table also illustrates the extensive and often confusing range of names used by the respective authors to refer to the nociceptors. Common characteristics are, however, apparent when the nociceptors are classified on the basis of their stimulus-specificity. Thus there can be distinguished three broad classes: mechanical nociceptors (which respond normally only to intense mechanical stimuli), mechanothermal nociceptors (which respond only to intense mechanical and thermal stimuli), and thermal nociceptors (which respond only to intense thermal stimuli). The characteristics of these three classes will now be briefly

discussed; data for each study cited is contained in table 1:1.

Mechanical nociceptors

These are variously known as high threshold mechanoreceptors (HTMs) (Fitzgerald and Lynn, 1977), high threshold mechanical fibres (Georgopoulos, 1976), moderate pressure receptors (Burgess and Perl, 1967) and low sensiti vity mechanoreceptors (Burgess and Perl, 1967). They are innervated by both unmyelinated and myelinated fibres. The myelinated fibres conduct mainly in the A-delta conduction range (5-30 m/sec), but several investigators report an overlap into the lowest end of the A-beta range (35-65 m/sec) e.g. Perl (1968) (monkey), Burgess, Petit and Warren (1968) (cat) and Lynn and Carpenter (1982) (rat). C-fibre innervated units have been reported infrequently, and little detailed information is available regarding their characteristics.

The receptive field of a mechanical nociceptor typically consists of several (1-20) excitable spots, each spot being <1mm^2 (Burgess and Perl, 1967; Dubner, Price , Beitel and Hu, 1977; Lynn and Carpenter, 1982). The skin between and surrounding these spots is relatively insensitive to stimulation. The area occupied by the receptive field varies from 15mm^2 (Campbell, Meyer and LaMotte (1979) up to 4.1 cm^2 (Fitzgerald and Lynn, 1977). Non-spot-like receptive fields

were reported by Bessou and Perl (1969) for C-fibre innervated mechanical nociceptors.

The mechanical thresholds, measured with von Frey hairs, range from 10mg (Perl, 1968) to 125g (Dubner et al, 1977) for the A fibre units, and from 0.6g (Bessou and Perl, 1969) to >45g (Bessou and Perl, 1969) for the C-fibre units. Several authors have reported a tendency in the A fibre units for the more sensitive units to have faster conducting axons (e.g. Burgess and Perl, 1967; Perl, 1968). The response (discharge frequency or number of spikes per stimulus) of the mechanical nociceptors is proportional to the stimulus intensity (Fitzgerald and Lynn, 1977; Campbell, Meyer and LaMotte, 1979). There is an overlap between the lower end of the threshold range for the mechanical nociceptors and the upper end of the threshold range of the low threshold mechanoreceptors. The crucial distinction between the two types of receptor is that although the threshold may be in the innocuous range, the maximal discharge of the mechanical nociceptors is produced by a stimulus which damages the skin (e.g. Perl, 1968). The mechanical nociceptors can therefore differentiate a noxious from a non-noxious stimulus. The low-threshold mechanoreceptors are maximally excited by innocuous stimuli, increase of intensity to noxious levels producing no further increases in discharge (Perl, 1968). They are therefore incapable of differentiating a noxious from an

innocuous stimulus.

The mechanical nociceptors do not respond to cooling and are not initially sensitive to heating. It has, however, been reported that some myelinated mechanical nociceptors do respond to heat (50-55°C) after repeated (2-6 times) heating to this intensity (Fitzgerald and Lynn, 1977; Lynn and Carpenter, 1982; Dubner et al , 1977). The term sensitization has been used by these authors to describe this phenomenon.

(b) Mechanothermal nociceptors

This class can be subdivided into three groups, again on the basis of stimulus-specificity. These three groups are (i) mechanical and heat nociceptors, (ii) mechanical, cold and heat nociceptors and (iii) mechanical and cold nociceptors.

(i) Mechanical and heat nociceptors

This group, the most frequently reported and intensely studied group of nociceptors, has an extensive and sometimes confusing range of names. They have been termed thermal nociceptors (Iggo and Ogawa, 1971), heat receptors (Beck, Handwerker and Zimmerman, 1974), heat sensitive myelinated fibres (Beck et al, 1974), A-delta heat nociceptive afferents (AHNs) (Dubner et al, 1977), mechanothermal nociceptive afferents (LaMotte and

Campbell, 1978), C-fibre nociceptive afferents (Meyer and Campbell, 1981a), high threshold mechanothermal fibres (Georgopoulos, 1976), A-fibre and C-fibre mechanoheat nociceptors (AMHs and CMHs respectively) (LaMotte, Thalhammer, Torebjork and Robinson, 1982) polymodal receptors (Torebjork and Hallin, 1976) and polymodal nociceptors (Bessou and Perl, 1969). To avoid any ambiguity, this group of nociceptors as a whole will be referred to as mechanoheat nociceptors. They are innervated by both unmyelinated and myelinated afferent fibres. The C-fibre innervated mechanoheat nociceptors have been described in the limb skin of human, monkey, cat, rabbit, and rat, and in the skin of the rabbit ear, rat tail and monkey face. They comprise a large proportion of the total afferent C-fibre population, i.e. 50% in cat, 85-90% in monkey, and 100% in human (Dubner and Bennett, 1983). The receptive fields of the C-mechanoheat nociceptors vary in size and organisation, both within and between species. They have been reported as single spots <1mm in diameter in the rabbit (Lynn, 1979), areas up to 35 mm^2 in the monkey (Meyer and Campbell, 1981a), and multiple spots in a relatively insensitive area in human and monkey (Van Hees and Gybels, 1972; Beitel and Dubner, 1976a). Mechanical thresholds range from 0.07g on the monkey face, (Beitel and Dubner, 1976a) up to 45g in the cat (Bessou and Perl, 1967). Heat thresholds range from 30 °C in the rat (Fleischer, Handwerker and Joukhadar, 1983), to 62.8°C in the rabbit (Lynn, 1979). The mechanical and thermal threshold

ranges are wide, but the maximum response in all the investigations cited is to stimuli of noxious intensity.

Bessou and Perl (1969) coined the term "polymodal nociceptor" to refer to cat C-fibre mechanoheat nociceptors which responded also to topical application of irritant chemicals (dilute acids) to the skin. The term polymodal nociceptor has been used subsequently by many investigators to refer to the nociceptors they have studied. The common use of this term is sometimes innaccurate and misleading, as only a few of the investigators who have used the term have actually tested chemical stimuli on the receptors involved. Excitation of mechanoheat receptors by chemical irritants applied to the skin has been observed in human (Van Hees and Gybels, 1972), monkey leg (Kumazawa and Perl, 1977) and face (Beitel and Dubner, 1976a), and rat leg (Lynn and Carpenter, 1982). In these cases, the term polymodal nociceptor is an accurate description of the receptor involved, in Bessou and Perl's (1969) original sense of the term.

Repetitive heat stimulation of the C-fibre mechanoheat nociceptors can produce a modification of the original heat-induced response. This modification can be either sensitization or depression. Sensitization was first reported by Bessou and Perl (1969) in the cat. It has subsequently been described in rat (Lynn and Carpenter, 1982), rabbit (Lynn,

1979), monkey (Beitel and Dubner, 1976 a, b) and man (Lamotte et al, 1982). This phenomenon generally consists of a decrease in the heat threshold, an increase in response (discharge frequency or number of impulses elicited per stimulus) to subsequent identical heat stimuli, and the development of a spontaneous discharge. Beitel and Dubner (1976a,b) also report a low-frequency (0.2-5ips), short-lived (1min to several mins) afterdischarge in association with the above response modifications. The extent of the modifications is reported to depend upon the intensity and duration of the heating, so that, for instance, Bessou and Perl (1969) found that a 5°C decrease in threshold occurred for the majority of their polymodal units after a 50 to 60 sec. exposure to heat 5°C above the original threshold. Depression or fatigue resulting from repetitive heat stimulation consists of an increase in heat threshold and a decrease in response to subsequent heat stimuli. LaMotte and Campbell (1978) reported that this occurred in response to heat stimuli of a range of intensities (45, 47, 49 and 53°C), with a stimulus duration of 35 sec. and an interstimulus interval of 25 sec. This protocol resulted in a 60% decrease in the number of impulses elicited between the first and the sixth application of an identical stimulus. LaMotte and Campbell's (1978) general conclusion was that the response to a heat stimulus was inversely related to the intensity, number and delivery rate of preceding heat stimuli. An extreme form of depression, a long lasting (hours) inactivation of

C-mechanoheat nociceptors, was noted by Beitel and Dubner (1976,a,b) after heat stimulation at >55°C for 5 sec, and by Bessou and Perl (1969) and Kumazawa and Perl (1977) after prolonged heating at 60°C. This inactivation was thought by them to be due to direct heat damage to the receptor itself.

Heat sensitization and depression appear to be variable and, to some extent, unpredictable phenomena so that, for example, repeated heating can sometimes lead to sensitization followed by depression (Beitel and Dubner, 1976a; Kumazawa and Perl, 1977). The range of effects reported in the literature indicate that the type and extent of the stimulus modification depends on a wide variety of factors, i.e. species, skin type and location and stimulus parameters such as intensity, duration, number, and delivery rate, all of which vary from one laboratory to the next.

The A-fibre mechanoheat nociceptors are innervated mainly by fibres which conduct in the A-delta range, with one report of overlap into the A-beta range (Campbell et al, 1979). Receptive field size and organisation is as described for the C-fibre units. Mechanical thresholds are reported to vary from 0.2g (monkey face, Dubner et al, 1977) to > 50g (applied using a $1mm^2$ probe in cat, Beck et al, 1974). Heat thresholds vary from 37°C (monkey face, Dubner et al, 1977) to >53°C (monkey hand, Campbell et al, 1979). Topical application of irritant

chemicals has been reported once (human, Adriaensen et al, 1983) and it had an excitatory effect. Heat sensitization by repetitive heat stimulation has been described (Campbell et al, 1979; Dubner et al, 1977).

(ii) Mechanical, heat and cold nociceptors

Nociceptors of this type, innervated by C-fibres, were first reported by Iggo (1959) in the cat. Iriuchijima and Zotterman (1960) and Witt and Griffin (1962) subsequently reported them in the rat, and termed them non-specific C-fibres. The C-fibre innervated type have since been described in the monkey by Georgopoulos (1976), LaMotte and Campbell (1978) and LaMotte and Thalhammer (1982). A-fibre innervated mechanical/heat/cold nociceptors have been reported in the monkey by Georgopoulos (1976) and LaMotte and Thalhammer (1982). Receptive fields have been described for both A- and C- fibre mechanical/heat/cold nociceptors in the monkey only. Georgopoulos (1976) described two types of receptive fields, $<5mm^2$ with 1-2 sensitive spots, or 9- $225mm^2$ with 2 or more spots (both types innervated by both A and C fibres). LaMotte and Campbell (1978) describe the receptive fields as elliptical, mean area of 18.9 mm^2 (both fibre types). Mechanical thresholds for both A- and C- fibre types were reported as 51mN (approx. 5g) (median) in the monkey (LaMotte and Campbell, 1978). Iggo (1959) reports a threshold of >5g for the cat C-fibre type. Heat thresholds of 41-53°C were reported for the monkey A-fibre

type (Georgopoulos, 1976), and a slightly lower range of 39-51 °C for the monkey C-fibre type (LaMotte and Thalhammer, 1982). For the cat and rat C-fibre types, heat thresholds were 55°C and >50°C respectively (Iggo, 1959; Iriuchijima and Zotterman, 1960). Cold thresholds were determined accurately only for the monkey. They were 31°C - ice for the A fibre type and 25°C - ice for the C fibre type (Georgopoulos, 1976).

(iii) Mechanical and Cold Nociceptors

Mechanical/cold nociceptors were first reported by Iggo (1959) in the cat. They were C-fibre innervated, with receptive fields of <5 x 5 mm, mechanical threshold >5g and a cold threshold of <17°C. They have been subsequently described only in the monkey by Georgopoulos (1976), LaMotte and Campbell (1978) and LaMotte and Thalhammer (1982). These three papers describe both A- and C- fibre innervated units. Receptive fields were described by Georgopoulos . For both A and C - fibre types there were two sorts, <5mm^2 with one or two sensitive spots, or 9-225mm^2 with 3 or more spots. Mechanical thresholds for both A and C fibre types were 51mN (approx. 5g) (LaMotte and Thalhammer, 1982). Cold thresholds, measured by Georgopoulos, were 31°C-ice for the A fibre type and 25°C - ice for the C-fibre type.

(c) Thermal nociceptors

Nociceptors responding only to thermal stimulation have been reported infrequently. They can be divided into three classes, those responsive to heat, those responsive to cold, and those responsive to both heat and cold.

Heat nociceptors have been described in the cat by Beck et al (1974), where they were innervated by C-fibres ("C-heat receptors"), and in the monkey by Georgopoulos (1976), where they were innervated by both A and C fibres ("high threshold thermal fibres"). Receptive fields were not described in either species. Cat heat thresholds were 40-55 °C (Beck et al, 1974). Monkey A-fibre heat thresholds were 41-53 °C, and C-fibre heat thresholds were 40-55°C (Georgopoulos, 1976).

Cold nociceptors have been described by Thalhammer and LaMotte (1982) in the monkey and the cat ("high threshold cold receptors"). One unit only was described for the cat; this was innervated by an A-delta fibre. In the monkey the cold nociceptors were innervated by both A-delta and C fibres. The cat receptive field was 1 mm^2. The monkey C-fibre units had receptive fields of 8-55mm^2, the monkey A -fibre units 4-145 mm^2. Cold threshold in the cat was 23°C. The monkey C -fibre threshold (1 unit) was 19°C, the monkey A fibre thresholds were 19-26°C.

Heat and cold nociceptors have been described by Georgopoulos (1976). They were innervated by either A-delta or C fibres. Receptive fields were not described. Heat thresholds were >41 °C for the A-delta fibre type, and 43-53°C for the C fibre type. Cold thresholds were <31 °C for the A-delta fibre type and 25°C - ice for the C fibre type.

(d) Morphology of the nociceptor

It is generally held that the anatomical substrate of nociceptors consists of epidermal free nerve endings. Direct evidence relating to the morphology of nociceptors was obtained by Kruger, Perl and Sedivec (1981), for the A-delta mechanical nociceptor in the hairy skin of the cat. After marking the responsive punctate spots in the receptive fields of several of these units, Kruger et al (1981) subjected these small skin areas to histological analysis. They consistently found, in association with each marked area, a thinly myelinated axon which lost its myelin sheath at the dermal papillary layer. The unmyelinated axon, covered with Schwann cell processes, penetrated the epidermal basal lamina. At the site of penetration the axoplasm was observed to contain both clear and dense core vesicles. At its ending within the basal epidermal layer the axon was surrounded by keratinocytes and lost its Schwann cell sheath. The finding of this structure in the

marked areas and its rarity in the surrounding skin strongly suggests that it is the anatomical substrate for the A-delta mechanical nociceptor.

2. Avian cutaneous nociceptors

Several authors have reported the presence of avian cutaneous receptors which responded to intense mechanical and/or thermal stimuli in birds. The species and areas of integument studied were chicken beak (Roumy and Leitner, 1973) and tongue (Kitchell, Strom and Zotterman, 1959), duck beak (Gregory, 1973; Leitner and Roumy, 1974b) and wing (Dorward, 1970), goose beak and facial skin (Gottschaldt, Fruhstorfer, Schmidt and Kraft, 1982) and pigeon wing (Necker 1979; Necker and Reiner, 1980).

(a) Chicken nociceptors

Afferent responses to potentially noxious stimulation have been recorded in the beak (Roumy and Leitner, 1973) and tongue (Kitchell et al, 1959) of the chicken but paucity of data restricts any conclusions about the classification of the receptors involved. For example, Roumy and Leitner (1973) recorded six units from the upper beak which responded with a SA discharge to the application of a crocodile clip to the dorsal and ventral side of the beak, and to 250g. force. No

further data on these units was reported. However, they also reported that some of the vibration sensitive mechanoreceptors in the beak gave a SA response to forces of >100 g., raising the possibility that the response to such mechanical stimulation could be a non-specific response.

Kitchell et al (1959) recorded multi-unit responses to heating the tongue of the chicken and pigeon above 44 C °and 45 °C respectively, with a contact thermode. The response to the first application of this stimulus was greater than that to successive applications.

More data are required to define the stimulus-response characteristics of receptors responding to potentially noxious stimuli in the chicken, to unequivocally distinguish them from other receptor types.

(b) Nociceptors in other avian species

Unequivocal evidence for specific nociceptors has been obtained in the pigeon (Necker, 1979;Necker and Reiner, 1980). They reported 10 units which responded to noxious heat stimulation of the feathered skin of the pigeon wing. They used a controlled and reproducible heat stimulus, namely a water perfused contact thermode, the temperature of which was measured by a thermistor on the thermode surface. All 10 units

responded to temperatures >45°C with a maintained discharge to suprathreshold stimuli lasting 1-2 minutes. The mean heat threshold, measured for 8 units, was 47.1± 1.4°C (s.d.). The units were not spontaneously active, and none responded to radiant cooling to 23°C, which indicates that the heat response was not a 'paradoxical' discharge from the cold receptors known to be present in the pigeon wing (Necker and Reiner, 1980) and beak (Necker, 1972). Necker and Reiner (1980) obtained quantitative heat stimulus -response data for 3 of the heat-sensitive units, by increasing the thermode temperature from 42 to 52°C in a stepwise manner, each step lasting 2 mins. All 3 units displayed an increase in firing rate with increase in temperature up to a maximum of 2 ips at 52°C, the resulting stimulus intensity - response curve being positively accelerating. Although specific warm receptors are present in the pigeon beak (Necker, 1972,1973), it is unlikely that the heat -sensitive units reported by Necker and Reiner (1980) were warm receptors, because the heat-sensitive units did not discharge below 45°C, in contrast to the warm receptors which are maximally activated at lower temperatures and displayed an increase in discharge rate from 0 to 70 ips over the range 20 to 44 °C. Necker and Reiner (1980) tested 5 of the 10 heat -sensitive units with a presumed noxious mechanical stimulus, pinching the skin with forceps. Three units were excited by this stimulus. The possibility that the nociceptors discharges could have been spurious responses from mechanoreceptors can

be discounted, as the RA mechanoreceptors present in the wing were not excited by heating or cooling (Necker and Reiner, 1980). The discharge of the SA mechanoreceptors was inhibited by skin heating from 43 to 50°C, and transiently increased by skin cooling from 43 to 25°C (Necker and Reiner,1980).

The heat response characteristics of the nociceptors described above are similar to those described for mammals, with respect to the heat thresholds and the shape of the heat intensity-response curves. The receptive fields of the avian nociceptors were noted for two units by Necker and Reiner (1980) and were described as spotlike. No measurements of conduction velocity of the afferent fibres were made, but an electron microscopic study of the fibre composition of the nerve involved revealed a strong bias towards the smaller fibres (20% C fibres, 70% A-delta fibres, 10% A-beta fibres) (Necker and Reiner, 1980).

Several other authors have described afferent responses to intense mechanical and or thermal stimuli in birds, but paucity of data precludes a definitive classification of the receptors involved. For example Dorward (1970) and Gregory (1973), recorded primary afferent activity from the wing (Dorward) and beak (Gregory) of the domestic duck and described receptors which they considered to be "pressure or nociceptive receptors" (Dorward, 1970) or "high threshold SA mechanoreceptors" to

which a nociceptive function was tentatively assigned (Gregory, 1973). Both authors reported high mechanical thresholds for these units, but this was not quantified. The adequate stimulus employed in both of these studies was pressure applied to the wing or beak by squeezing the organ between finger and thumb. The units described by Gregory (1973) were inactive in the absence of stimulation, and were insensitive to vibratory or thermal stimuli. Receptive fields of the units in the wing were reported as "diffuse" (Dorward, 1970). Gregory (1973) reported some receptive fields (numbers and sizes not mentioned) on the ventral border of the beak.

Gottschaldt et al (1982) in their study of mechanoreceptors in the goose beak and facial skin, reported one receptor which they described as nociceptive. This responded to strong pinch of the upper eyelid, but no further characteristics were described.

Leitner and Roumy (1974) recorded one unit from the duck beak which responded to touching the beak skin with a hot copper tube (filled with water at 60°C). No response was elicited by cooling, or pinching the skin with an alligator clip, or applying pressure of 250 g/mm^2 with a perspex rod.

Gottschaldt et al (1982) recorded one unit in the goose beak which responded to cooling with ice and one which responded to

noxious radiant heat. Two of their nociceptive units had conduction velocities of <2 m/s. It is not clear however, whether these are the same two units.

To summarize, the data obtained by Necker and Reiner (1980) from the pigeon wing satisfy the criteria for classification as nociceptors. The rest of the published material on avian species indicates that receptors sensitive to potentially tissue-damaging stimuli exist, but paucity of data precludes any conclusive statement about their nature.

However, Necker (1977) observed that heating the beak of the lightly anaesthetized pigeon to 45°C resulted in repeated openings of the beak and movements of the tongue, which he considered to represent a defence reaction. This observation strongly suggests that information regarding potentially noxious heat is transmitted from the beak to the central nervous system.

IV. PATHOLOGY AND PATHOPHYSIOLOGY OF THE AMPUTATED BEAK STUMP

No systematic studies have been made of the pathology or pathophysiology of the beak stump which remains after beak trimming. Incidental observations (Andrade and Carson, 1975; Hubrecht, 1979) indicate that the beak can regenerate. For example, Andrade and Carson (1975) mention that "birds debeaked at six and eight weeks old completely regrow their beaks and developed a noticeable overgrowth and exaggerated curvature of the upper beak". Hubrecht (1979) states that, after removal of one third of the upper beak, "three of the birds showed complete regrowth, in three of the birds regrowth occurred on one side of the beak only, giving a cross bill effect, whilst in the other three no regrowth was found". No further information has been found regarding beak regeneration.

V. AIMS OF THIS STUDY

Beak trimming constitutes a tissue damaging mechanical and thermal stimulus. In principle this may bring about pain and suffering in the bird, provoked by both acute and chronic activity in sensory nerves. The experimental part of this thesis was designed to search for such activity, using electrophysiological techniques.

Figure 1:1. The skeleton of the head of the chicken.

A. lateral aspect of skull
B. lower beak, dorsal aspect
C. upper beak and skull, ventral aspect

a - os occipitale; b - basisphenoid and b' - presphenoid of the os sphenoidale; c- os ethmoidale; d - os parietale; e - os frontale; f - os oticum and f'- pars squamosa of the os temporale; g - os lacrimale; h - os nasale; i - os intermaxillare; k - os maxillare; l - os zygomaticum; m- os palatinum; n- os pterygoideum; o - vomer; p - os quadratum; q - mandible (dorsal view)

1 for. occipitale magnum; 2 condylus occipitalis; 3 for. n. hypoglossi; 4 for. n. vagi et glossopharyngici; 5 for. or canalis caroticus; 6 for. jugulare; 7 porus acusticus ext.; 8 Eustachian tube; 9 art. pterygoideosphenoidea; 10 septum interorbitale; 11 pars frontalis; 12 pars orbitalis and 13 pars nasalis of the os frontale; 14 proc. orbitalis of the os temporale; 15 proc. zygomaticus s. suprameaticus; 16 for. opticum; 17 for. olfactorium; 18 proc. lacrimalis; 19 proc. frontalis with 19' its squamous suture; 20 proc. intermaxillaris and 21 proc. maxillaris of the os nasale; 22 proc maxillaris; 23 proc. palatinus and 24 proc. frontalis of the os intermaxillare; 25 proc. oticus; 26 proc. articularis and 27 proc. orbitalis of the os quadratum ; 28 angular bone with 28' proc. angularis int. and 28'' post.; 29 articular bone; 30 supra-angular bone; 31 dental bone; 32 art. quadratosquamosa; 33 art. quadratozygomatica; 34 art. quadratopterygoidea; 35 art. palatoptergoidea; 36 art. palatomaxillaris.

From Nickel et al, (1977)

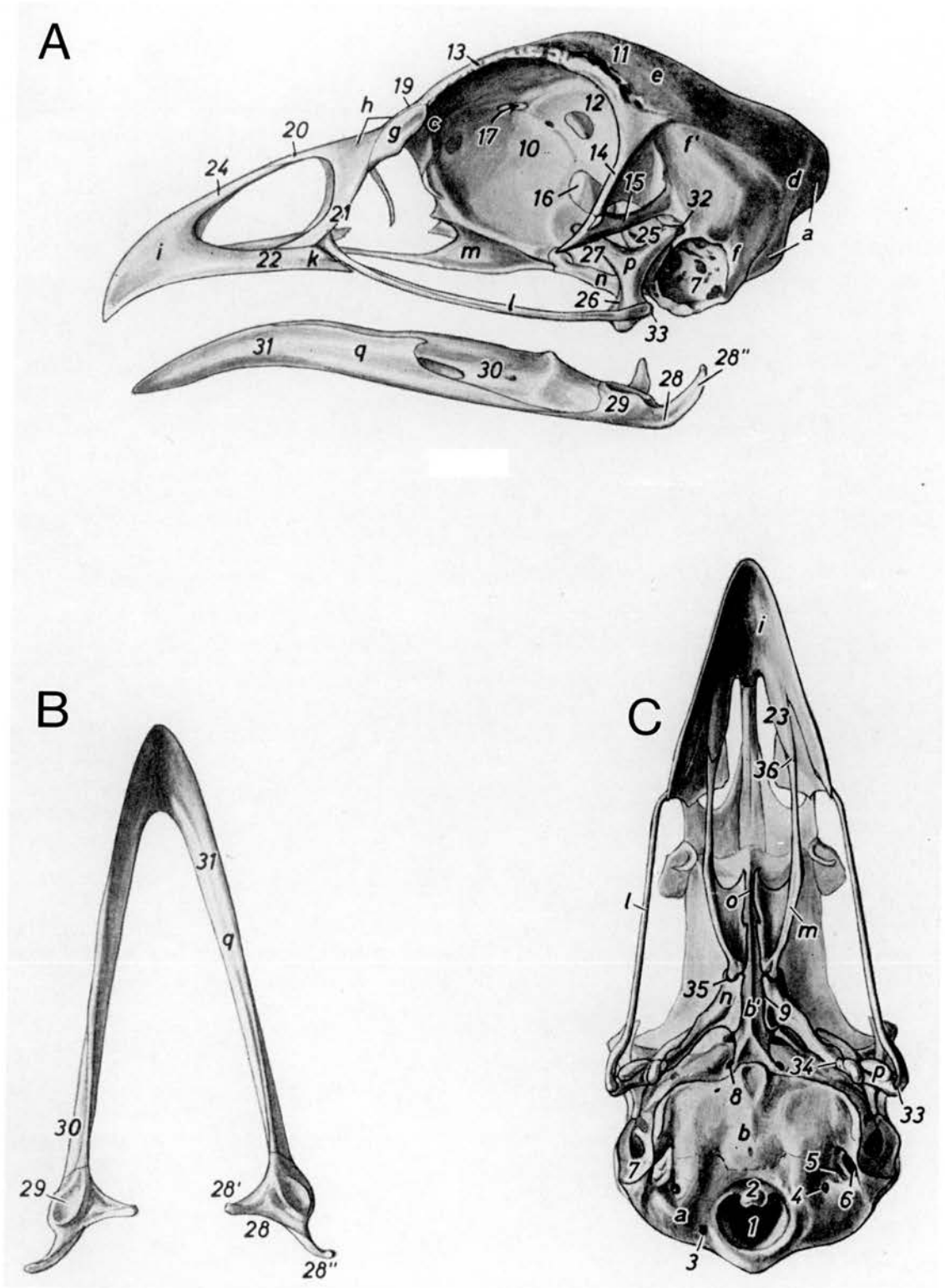
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Figure 1:2. The heads of the four domestic species mentioned in this thesis, illustrating general anatomical features

A. Chicken
B. Duck
C. Goose
D. Pigeon

a upper beak
a' horny plate at tip of beak (duck and goose)
a'' cere (pigeon)
b lower beak
c external nares
d external auditory meatus covered with feathers
e eye
f,g,h lower, upper and third eyelids
1 comb
2 ear lobe
3 wattle

(from Nickel et al, 1977)

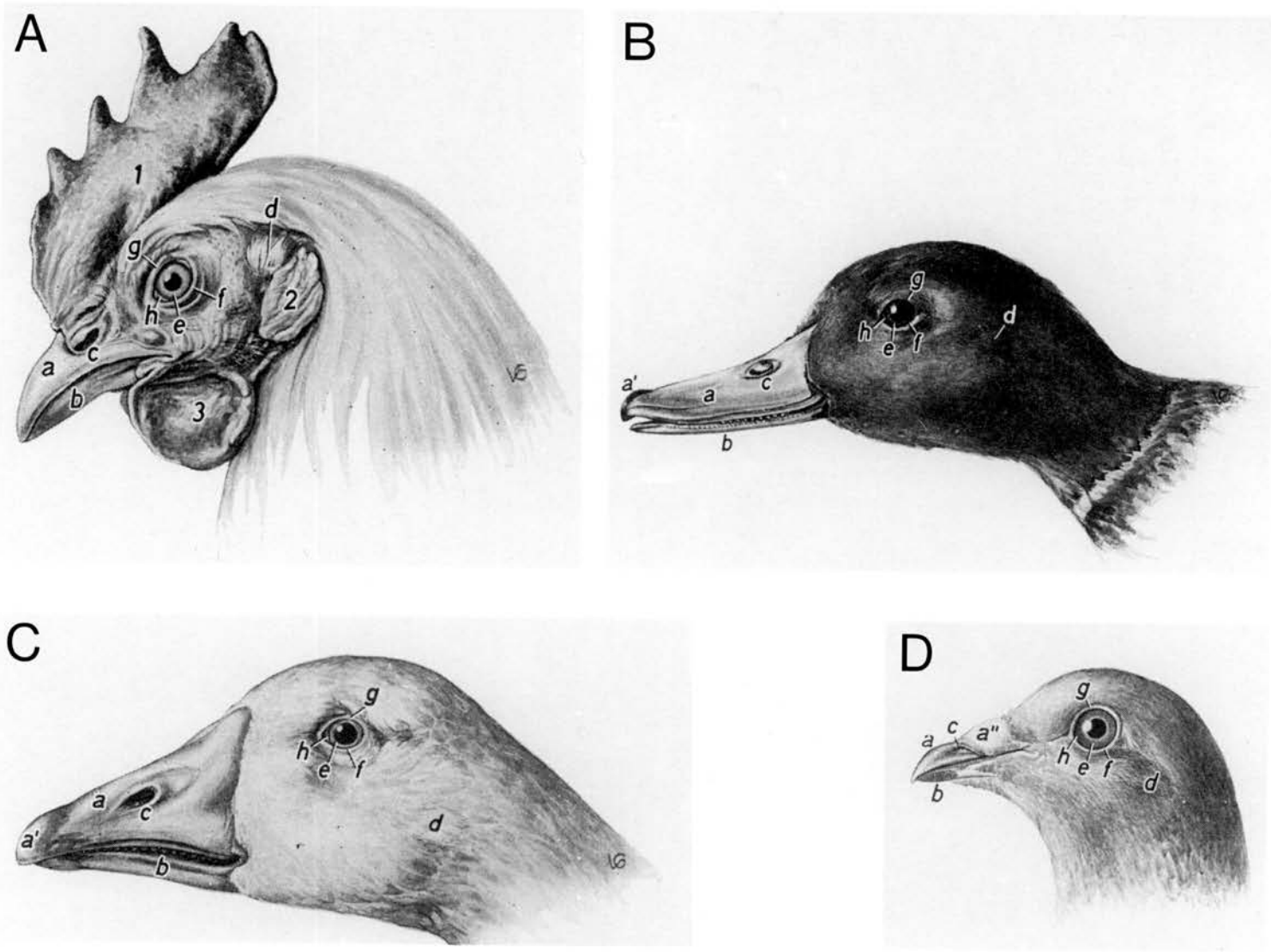
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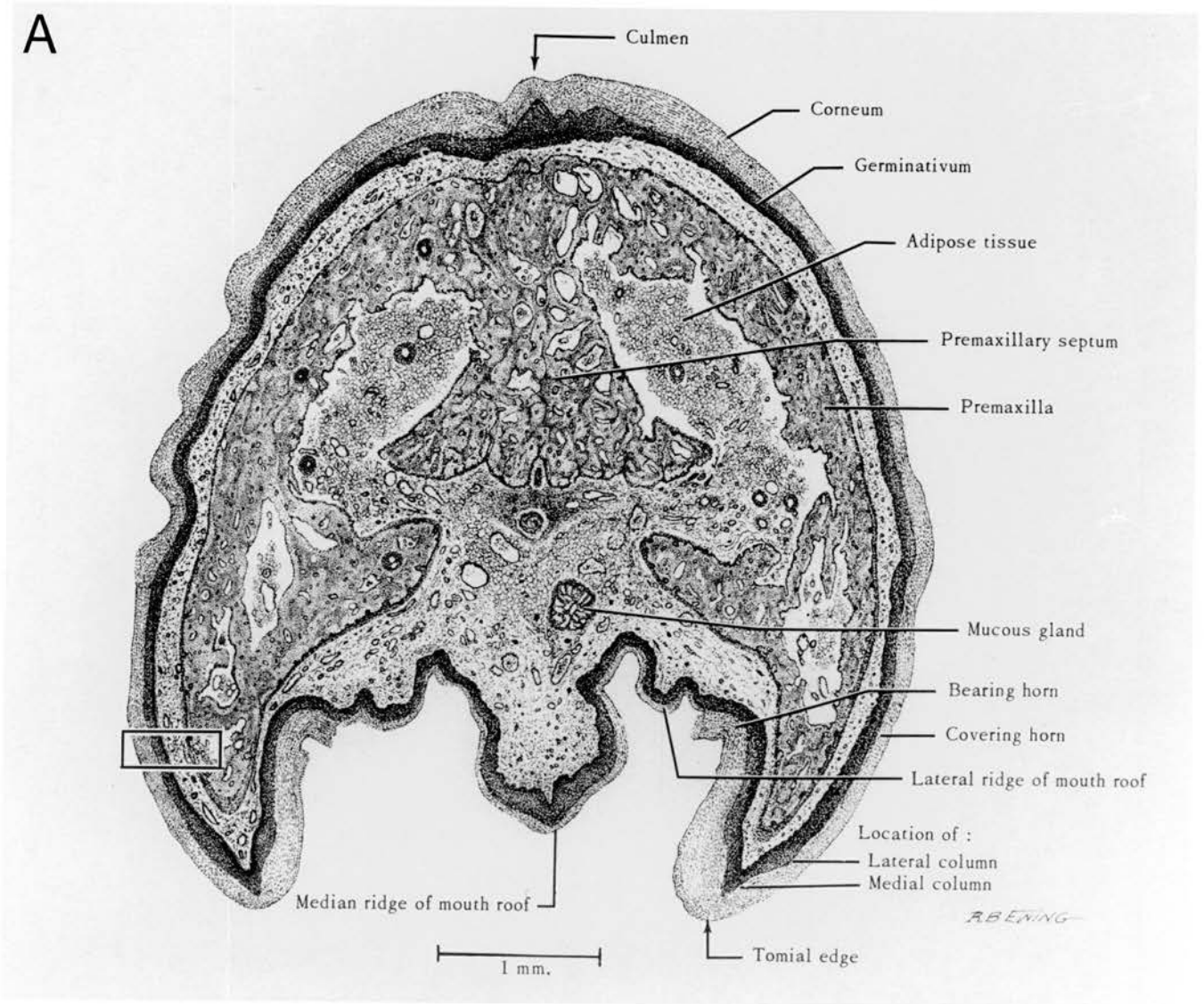
Figure 1:3.A. Transverse section through the upper beak near its anterior tip. The area enclosed by the rectangle is shown at higher magnification in B. Tissue taken from a single comb White Leghorn Chicken, 40 days old. Bouin fixation, Haematoxylin and Eosin staining.

(From Lucas and Stettenheim, 1972).


A
Culmen
Corneum
Germinativum
Adipose tissue
Premaxillary septum
Premaxilla
Mucous gland
Bearing horn
Covering horn
Lateral ridge of mouth roof
Location of :
Lateral column
Medial column
Median ridge of mouth roof
Tomial edge
1 mm.
R.B.ENING

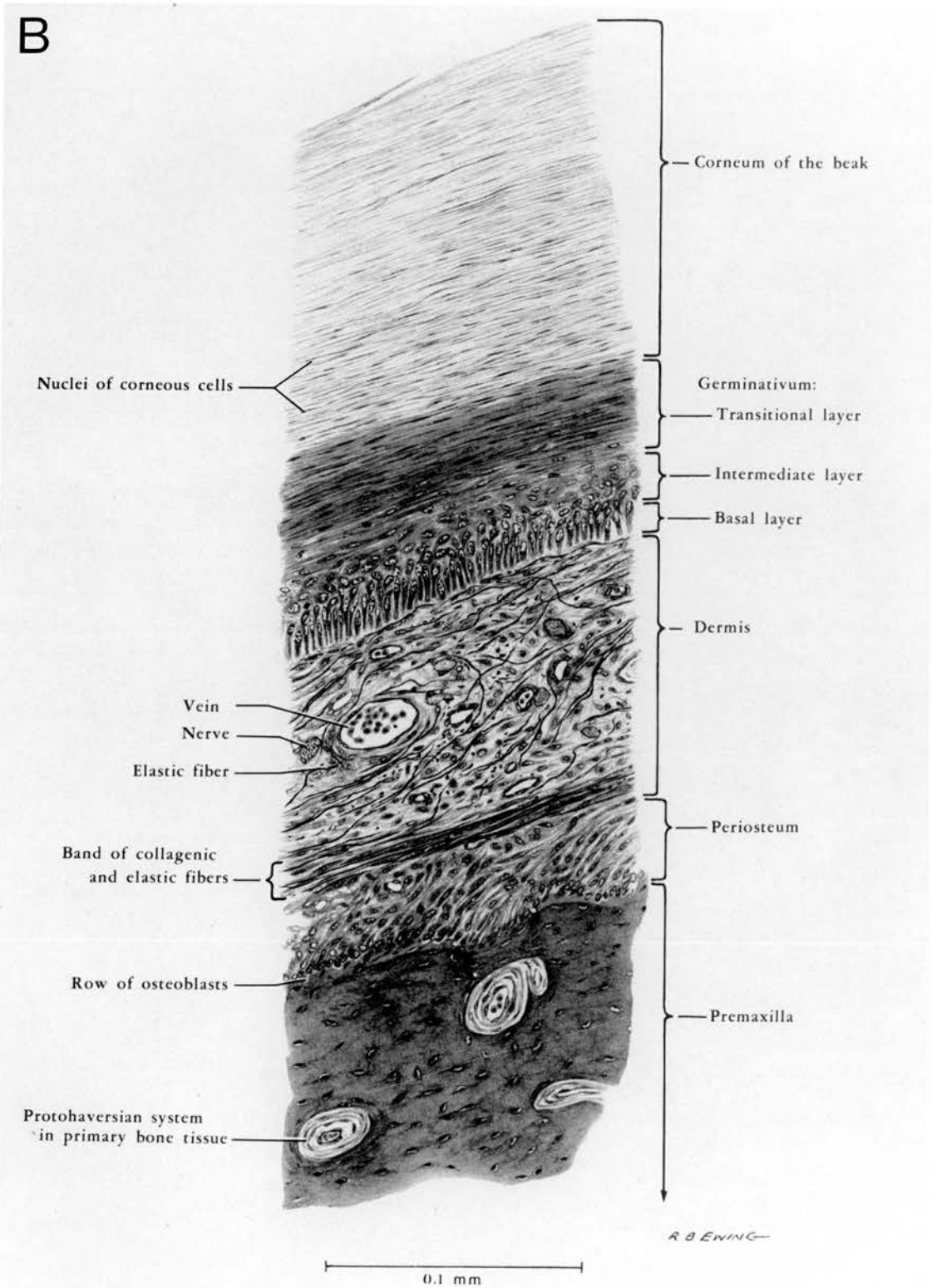

B
Corneum of the beak
Nuclei of corneous cells
Germinativum:
Transitional layer
Intermediate layer
Basal layer
Dermis
Vein
Nerve
Elastic fiber
Periosteum
Band of collagenic
and elastic fibers
Row of osteoblasts
Premaxilla
Protohaversian system
in primary bone tissue
R B ENING
0.1 mm

Figure 1.4. Diagrammatic illustration of the cranial nerves of the chicken. (After Schrader, 1970).

1 n. olfactorius; 2 n. trochlearis; 2 n. ophthalmicus; 4 nn. ciliares longi; 5 n. frontalis; 6 n. intratrochlearis; 7 r. nasalis int. lat.; 8 rr. nasales extt; 9 ganglion pterygopalatinum dors.; 10 rr. postganglionares ophthalmici; 10' rr. postganglionares glandulares; 11 rr. orbitales; 12 radix autonomica of the ganglion pterygopalatinum dors.; 13 radix autonomica of the ganglion pterygopalatinum ventr.; 14 n. ethmoidalis; 15 r. lat. rostri sup.; 16 r. med. rostri sup.; 17 n. opticus; 18 n. oculomotorius with its r. ventr., which gives off the radix parasympathica to the ganglion ciliare. (The r. dors is not shown); 19 ganglion ciliare; 20 nn. ciliares breves; 21 r. zygomaticotemporalis; 22 n. lacrimalis; 23 r. zygomaticofacialis; 24 n. maxillaris; 24' n. infraorbitalis; 25 rr. palprebrales; 26 r. nasalis int. dors.; 27 n. pterygopalatinus; 28 n. palatinus major; 29 n. nasalis cuad.; 30 r. nasalis int lat.; 30' r. nasalis int. med.; 31 rr. postganglionares pterygopalatini; 32 communicationes petrosomandibulares et petrosomaxillares; 33 chorda tympani; 34 n. pterygoideus lat.; 37 n. temporalis prof.; 38 n. buccalis; 39 n. lingualis; 39' n. sublingualis; 40 n. alveolaris mandibularis; 41 n. mylohyoideus; 42 r. angularis oris; 43 rr. medd. rostri inf.; 43' rr. latt. rostri inff.; 44 r. digastricus; 45 r. colli; 46 r. stylohyoideus; 47 n. glossopharyngeus; 48 r. pharyngeus rost.; 49 communicatio vagoglossopharyngea; 50 r. lingualis; 51 r. glandularis; 52 r. pharyngeus caud.; 53 its cranial, 54 its middle, 555 its caudal.

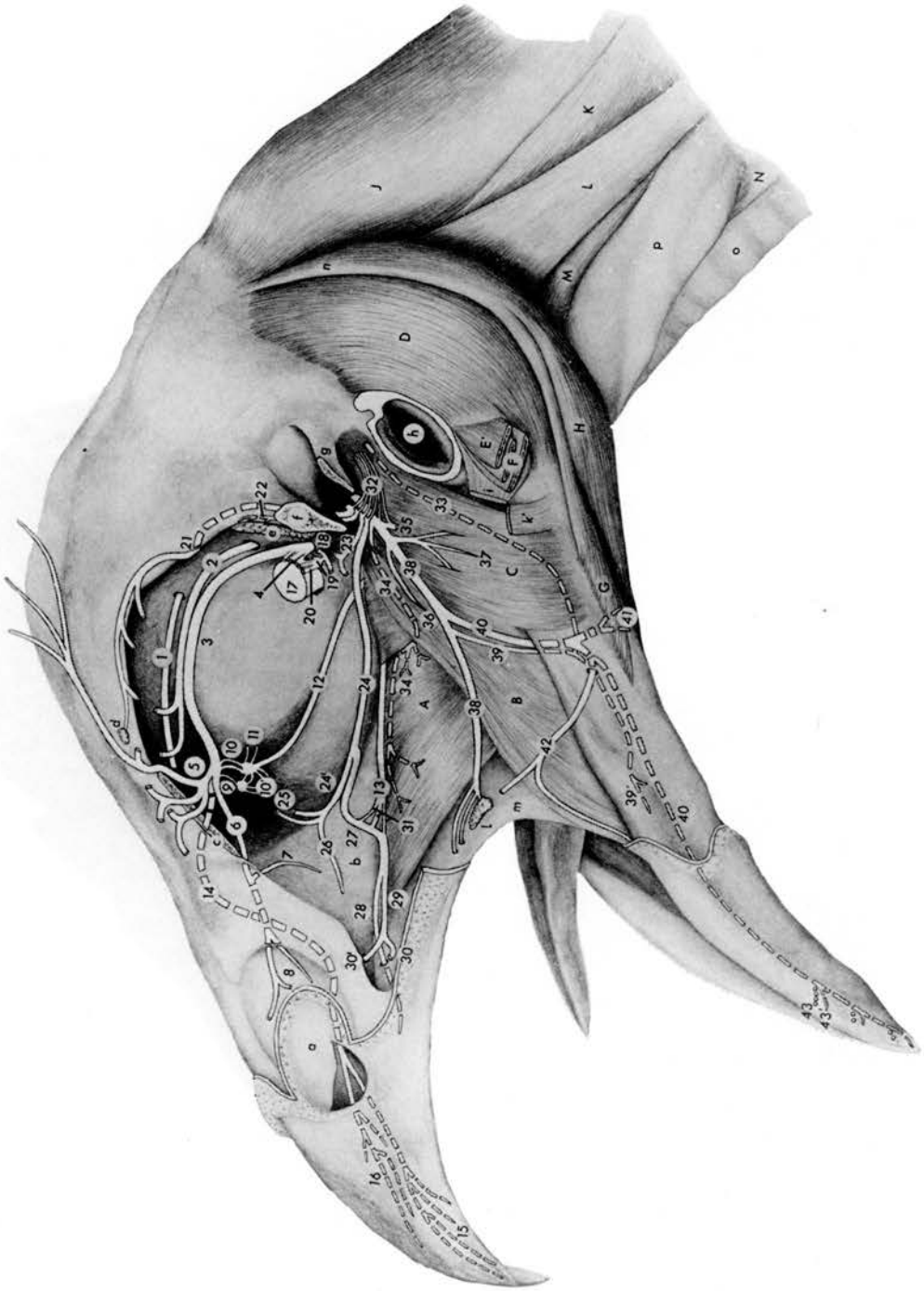


Figure 1:5. Diagrammatic three dimensional view of a Herbst corpuscle.

A. Light microscopic appearance

1 Myelinated afferent fibre
2 Outer capsule
3 Outer zone
4 Inner cone

B. Electron microscopic appearance of distal inner core, showing its appearance from the outside, and in cross section and longitudinal section.

5 lamellae of the inner core
6 nucleus of satellite cell
7 receptor axon
8 terminal enlargement of receptor axon
9 axon processes

(From Gottschaldt et al, 1982)

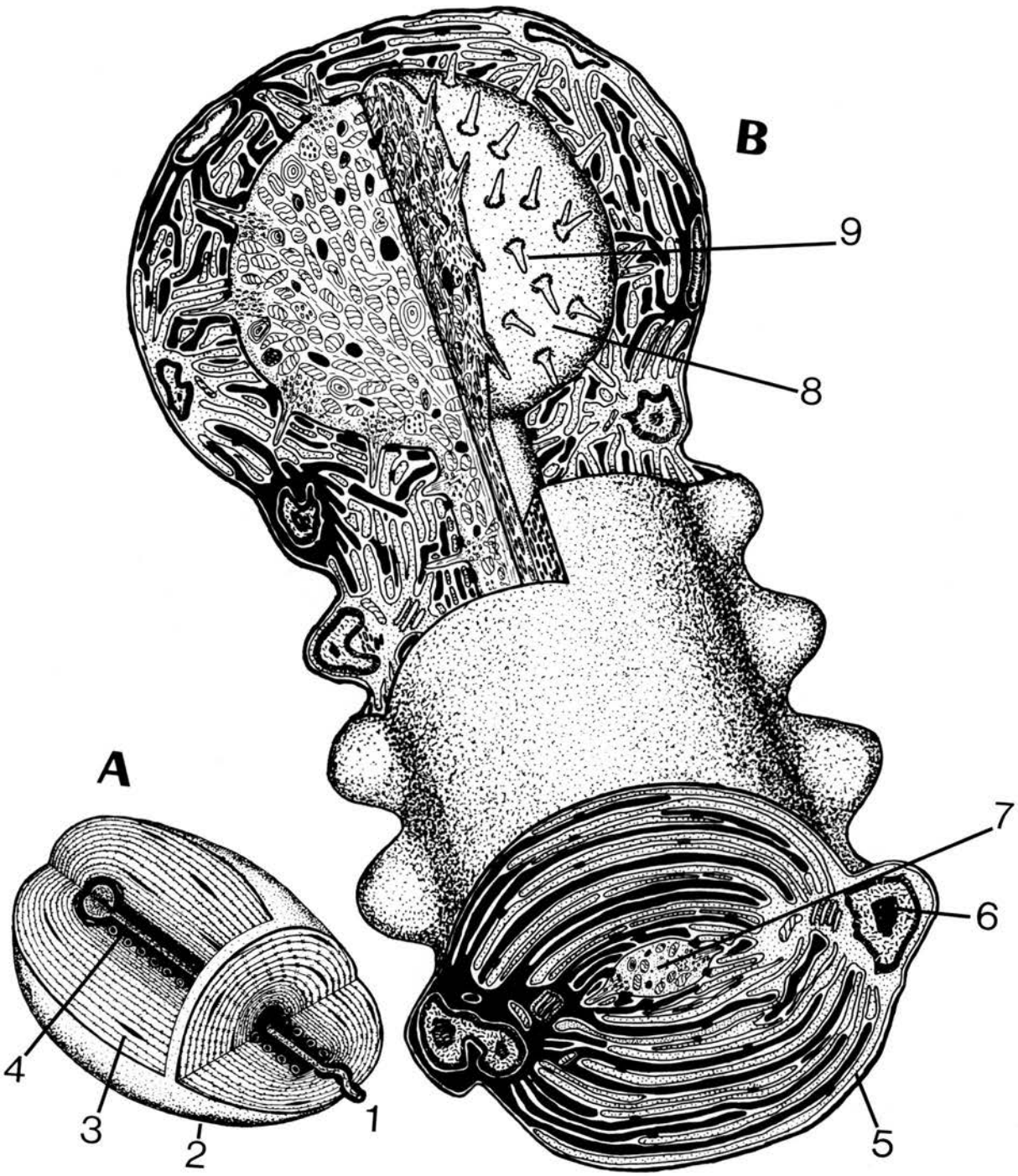

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Figure 1:6. Diagrammatic section through Grandry corpuscle with 2 cells

1. Afferent nerve fibre with sheath of Schwann and myelin
2. Schwann cell enclosed in basal membrane
3. Terminal disc
4. Specialized cell
5. Golgi apparatus
6. Granular endoplasmic reticulum
7. Osmiophilic granules
8. Filaments
9. Accumulation of glycogen granules
10. Fibroblasts forming incomplete capsule
11. Dermis

(from Halata, 1971)

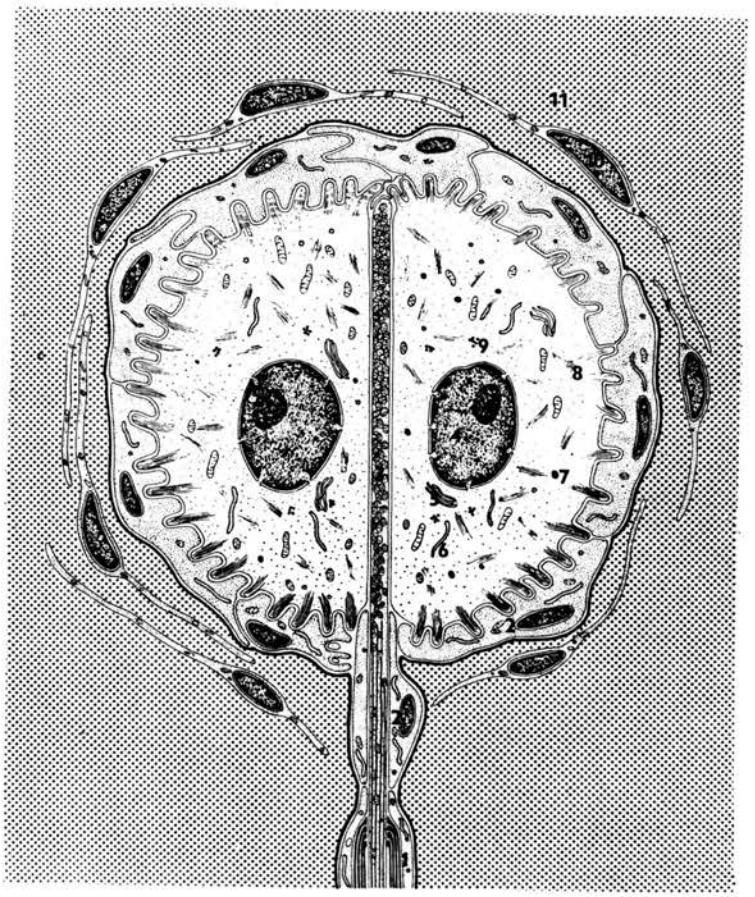
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Figure 1:7. Diagrammatic view of a tubule in the bill tip organ in the mandibular nail of the duck.

(From Berkhoudt (1980))

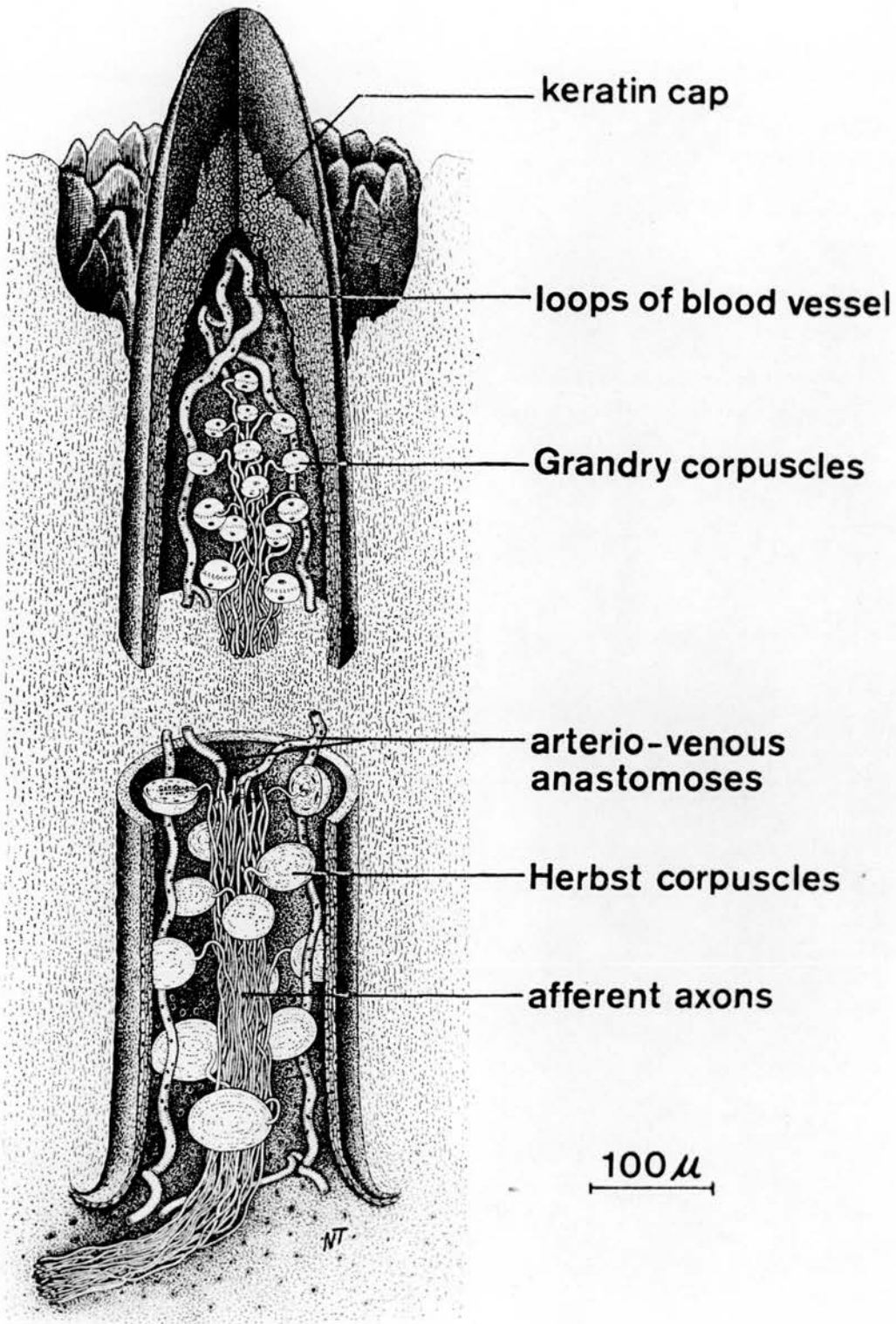

keratin cap
loops of blood vessel
Grandry corpuscles
arterio-venous anastomoses
Herbst corpuscles
afferent axons
100 μ
NT

Table 1:1. Physiological properties of mammalian nociceptors.

+ CHEM = unit responds to topically applied chemical irritants.

- = not reported

Mechanical thresholds were measured with von Frey hairs unless otherwise stated.

MECHANICAL NOCICEPTORS - A fibre innervated

HAIRY SKIN

Species	Skin region	Conduction velocity of afferent fibre (m/sec)	Stimulus threshold	Receptive field	Other comments	Terminology used by authors	Authors
Human	hand	mean 19.2 ± 7.2	8.8 - 221mN +CHEM	-		High threshold mechanoreceptor (HTM)	Adriaensen et al (1983)
Monkey	hand and leg	5 - 39	(i) 10mg - 800mg (ii) 600mg - 3g (iii) > 7.2 g	3 - 20 spots (<1mm²) in area of 10 - 20 mm²		(i) Moderate pressure receptor (ii)Low sensitivity mechanoreceptor (iii) Nociceptor	Perl (1968)
Monkey	face	mean 10.2 ± 7.2	0.2 - 125g median 1.3g	single spots 1 - 2mm diam.	20-25% responded to heat stim. after repeated exposure to heat > 50°C. thresholds 43 to 53°C (median 47°C)	HTM	Dubner and Hu (1977) Dubner et al (1977)
Cat	hind foot	-	>50g (1mm diam. probe)	-		HTM	Beck et al (1974)
Cat	hind leg	6 - 51	(i) 0.1 - 0.8g. (ii)0.5 - 3.3g. (iii) > 3.3g.	single spots (<1 mm²) in a field 2 - 5 cm. long by 1 - 2.5 cm. wide.		(i) moderate pressure receptor (ii)low sensitivity mechanoreceptor (iii)nociceptor	Burgess and Perl (1967)

Cat	foot	6 - 65	-	single spots in large area		(i) moderate pressure receptor (ii)low sensitivity mechanoreceptor (iii)nociceptor	Burgess et al (1968)
Cat	hind leg	6 - 42	Not determined Units responded to heavy pinch or pin-prick	-		Probable nociceptive receptor	Hunt and McIntyre (1960)
Cat	hind leg	5.5 - 49	8/13 units excited by 50mN	single spots in area of 0.4 - 4.1cm^2	77% responded to heat stim. after 2 - 6 trials of heat to 50-55°C	HTM	Fitzgerald and Lynn (1977)
Rabbit	hind leg and foot	5.0 - 32.5	37/38 units excited by 50mN	2 - 16 single spots in area of 0.18-2.6cm^2	40% responded to heat stim. after 2-6 trials of heat to 50 - 55°C	HTM	Fitzgerald and Lynn (1977)
Rat	hind leg and foot	3.6 - 40		2 - 7 single spots (<1mm. diam.)	1/6 units responded to heat (55°C)	HTM	Lynn and Carpenter (1982)

GLABROUS SKIN

Monkey	hand	5 - 39	(i) 10mg-800mg (ii)600mg-3g (iii)>7.2g	3-20 single spots < 1mm² in area of 10-20 mm²	(i) Moderate pressure receptor (ii) low sensitivitiy mechanoreceptor (iii) nociceptor	Perl (1968)
Monkey	hand	3 - 44	>9.4 bars	(i) <5mm², 1-2 spots (65% of units) (ii) 8 - 200 mm² 3 or more sensitive spots	High threshold mechanical fibre	Georgopoulos (1976)
Monkey	hand	2.9-42.7	4.1-14.5 bars (mean 9.7 bars)	15-80mm² area (mean 46 mm)	-	Campbell et al (1979)
Cat	hind foot	-	> 50g (1mm diam. probe)	-	HTM	Beck et al (1974)

MECHANICAL NOCICEPTORS - C fibre innervated

HAIRY SKIN							
Monkey	leg	< 1.5	-	-	1 unit	HTM	Kumazawa and Perl (1971)
Monkey	hand and forearm	< 2	-	-		-	LaMotte and Campbell (1978)
Cat	hind foot	< 2	>50g (1mm diam. probe)	-		HTM	Beck et al (1974)
Cat	hind leg	< 2.2	0.6 - >45 g	(i)1 x 2 mm (ii)>1 cm long several mm wide		HTM	Bessou and Perl (1969)
Rat	hind leg and foot	< 0.9	>40mN	-		insensitive C fibre unit	Lynn and Carpenter (1982)

MECHANICAL NOCICEPTORS - C fibre innervated

GLABROUS SKIN						
Monkey	hand	< 2	>9.4 bars	(i) < 5mm², 1-2 spots (65% of units) (ii) 8-200mm², 3 or more sensitive spots	High threshold mechanical fibre	Georgopoulos (1976)
Monkey	hand	< 2	-	-	-	LaMotte and Campbell (1978)
Cat	hind foot	< 2	>50g (1mm diam. probe)	-	HTM	Beck et al (1974)

HAIRY SKIN

MECHANOTHERMAL NOCICEPTORS (mechanical and heat) - A fibre innervated

Species	Skin region	Conduction velocity of afferent fibre (m/sec.)	STIMULUS THRESHOLD		Receptive field	Terminology used by authors	Authors
			mechanical	heat			
Human	hand	mean 19.2 ±7.2	8.8-221mN	>44°C +CHEM	-	Mechanothermo-receptor (MTR)	Adriaensen et al (1983)
Monkey	leg	3.9 - 6.8	not determined. Units responded to squeezing, pinching and pricking	>40°C	>3mm diam. uniformly sensitive	thermal nociceptor	Iggo and Ogawa (1971)
Monkey	leg	3.5 (1 unit)	-	>46°C	-	-	Kumazawa and Perl (1977)
Monkey	hand	5.2 - 53.3 (mean 31.1±1.5)	1.6 - 9.4 bars (mean 3.5±0.3 bars)	45 - >53°C (before sensitisation) <38 - 49°C (after sensitisation)	2.3 - 121mm² (mean 37+4mm²)	A fibre mechanoheat nociceptor (AMH)	Campbell et al (1979)
Monkey	hand, arm, leg, foot	mean 15.0±7.0	-	47->51°C	-	AMH	LaMotte et al (1982)
Monkey	face	mean 15.2±9.9	0.2 - 15.0g (median 0.4g)	37 - 47°C (median 43°C)	single spots 1 - 2 mm diam.	A heat nociceptive afferents (AHN)	Dubner et al (1977)
Cat	foot	-	>50g (1mm diam. probe)	-	-	heat sensitive myelinated fibre	Beck et al (1974)

GLABROUS SKIN

MECHANOTHERMAL NOCICEPTORS (mechanical and heat) - A fibre innervated

Species	Skin region	Conduction velocity of afferent fibre (m/sec.)	STIMULUS THRESHOLD		Receptive field	Terminology used by authors	Authors
			mechanical	heat			
Monkey	hand	3 - 39	>9.4 bars	41-53°C	(i) <5 mm^2, 1-2 spots. (ii) 9 - 225mm^2, 3 or more spots.	high threshold mechanothermal fibre	Georgopoulos (1976)
Monkey	hand	5.2-53.3 (mean 31.1±1.5)	1.6-9.4 bars (mean 3.5±0.3 bars)	45->53°C (before sensitisation) <38-49°C (after sensitisation)	2.3-121mm^2 (mean 37±4mm^2)	AMH	Campbell et al (1979)
Monkey	hand	mean 34.7±1.8	mean 3.6±0.26 bars	-	mean 37.3±2.8mm^2	AMH	Meyer and Campbell (1981b)
Monkey	hand	mean 22.2±13.0	-	47->51°C	-	AMH	LaMotte et al (1982)
Cat	foot	-	>50g (1mm diam. probe)	-	-	heat sensitive myelinated fibres	Beck et al (1974)

HAIRY SKIN

MECHANOTHERMAL NOCICEPTORS (mechanical and heat) - C fibre innervated

Species	Skin region	Conduction velocity of afferent fibre (m/sec.)	STIMULUS THRESHOLD		Receptive field	Terminology used by authors	Authors
			mechanical	heat			
Human	hand and foot	0.7 - 1.0	not measured units excited by firm pressure or pinprick	not measured units excited by radiant heat or burning match + CHEM	5-7 x 3-5mm (5 units) 1 x 1 mm (1 unit. >1cm² (1 unit)	-	Torebjork and Hallin (1974)
Human	hand and foot	0.4 - 1.8	0.7 - 8.5g	40-47°C +CHEM	single spots or several spots in a field of relative insensitivity	polymodal receptor	Torebjork and Hallin (1976)
Human	arm	0.66-1.1 (mean 0.89)	2.3 - 13.1g	not measured units excited by radiant heat +CHEM	6 - 9 mm diam. single spots in insensitive field	polymodal nociceptor	van Hees and Gybels (1972)
Human	hand	0.86-1.25	not measured units excited by squeezing or pricking	45°C	<5mm²	polymodal nociceptor	Gybels et al (1979)
Human	leg and foot	mean 0.85±0.17	-	41-43°C	-	C fibre mechanoheat nociceptor (CMH)	LaMotte et al (1982)

HAIRY SKIN

MECHANOTHERMAL NOCICEPTORS (mechanical and heat) - C fibre innervated

Species	Skin region	Conduction velocity of afferent fibre (m/sec.)	STIMULUS THRESHOLD		Receptive field	Terminology used by authors	Authors
			mechanical	heat			
Human	leg and foot	mean 0.84	-	41-43°C	-	CMH	Torebjork et al (1984)
Monkey	leg	<2.5	not measured units excited by squeezing, pinching or pricking	>40°C	>3mm diam. uniformly sensitive	thermal nociceptor	Iggo and Ogawa (1971)
Monkey	leg and arm	0.6 - 1.1	not measured units excited by pricking or pinching	40-46.5°C (mean 42.5°C)	(i)single spot 1-2mm^2 (ii)multiple spot-like areas 1-2 mm^2. (iii) areas up to 25mm^2 with heterogenous sensititvity	polymodal nociceptor	Croze et al (1976)
Monkey	leg	<1.5	6-26 g/mm (mean 11±7 g/mm)	42-55°C+CHEM	point-like, <0.5mm diam. or larger, ovoid 2-3mm diam.	polymodal nociceptor	Kumazawa and Perl (1977)
Monkey	face	0.5-1.2 (mean 0.82 ±0.17)	0.07-8.5g (median 1.2g)	38-49°C (median 46°C) +CHEM	single spots 2mm^2. 1 unit - 2 small zones separated by insensitive area. 1 unit field of 3 x 5 mm with 11 sensitive spots.	C polymodal nociceptor	Beitel and Dubner (1976)

HAIRY SKIN

MECHANOTHERMAL NOCICEPTORS (mechanical and heat) - C fibre innervated

Species	Skin region	Conduction velocity of afferent fibre (m/sec.)	STIMULUS THRESHOLD		Receptive field	Terminology used by authors	Authors
			mechanical	heat			
Monkey	hand	(i)mean 0.83±0.07 (ii)mean 0.82 ±0.03	(i)quickly adapting (QC) 2.11±0.21 bars (ii) slowly adapting (SC) 2.88±0.18 bars	(i)44.3±0.2°C (ii)45.1±0.4°C	23.1±2.0mm 29.9±2.6mm	C fibre nociceptive afferent	Meyer and Campbell (1981a)
Monkey	hand, arm, leg, foot	mean 0.8±0.1	-	39->51°C (median 45°C)	-	CMH	LaMotte et al (1982)
Cat	leg	0.4 - 1.1	0.2 - 45g	42 - 56°C +CHEM	single spot <2 mm²	polymodal nociceeptor	Bessou and Perl (1969)
Cat	leg and foot	0.66 - 1.0	>1.5g	>44°C	<5 x 5 mm	heat receptor	Iggo (1959)
Cat	foot	0.4 - 1.7	<300g/0.8mm²	40-55°C	-	C-heat receptor	Beck et al (1974)

HAIRY SKIN

MECHANOTHERMAL NOCICEPTORS (mechanical and heat) - C fibre innervated

Species	Skin region	Conduction velocity of afferent fibre (m/sec.)	STIMULUS THRESHOLD		Receptive field	Terminology used by authors	Authors
			mechanical	heat			
Rabbit	ear (perfused)	-	>20g/mm	40-60°C +CHEM	-	polymodal nociceptor	King et al (1976)
Rabbit	leg	0.72-1.13 (mean 0.90)	0.3-9mN (mean 3.1mN)	41.1-62.8°C (mean 52.5°C)	1 spot <1mm diam.	polymodal nociceptor	Lynn (1979)
Rat	foot and leg	0.4-0.8 (mean 0.67 ±0.08)	0.8->50mN	36-59°C (mean 47°C) +CHEM	single spot <2mm diam.	polymodal nociceptor	Lynn and Carpenter (1982)
Rat	leg	<2	14-400mN (median 52mN)	30-55°C	1-2mm²	CMH	Fleischer et al (1983)
Rat	tail	-	not measured units excited by pinching	>40°C	2-3mm²	-	Necker and Hellon (1978)

MECHANOTHERMAL NOCICEPTORS (mechanical and heat) - C fibre innervated

GLABROUS SKIN

Species	Skin region	Conduction velocity of afferent fibre (m/sec.)	STIMULUS THRESHOLD		Receptive field	Terminology used by authors	Authors
			mechanical	heat			
Human	hand	<1.5	not measured units respond to squeezing and pricking	45°C	<5mm²	polymodal nociceptor	Gybels et al (1979)
Monkey	hand	0.8-1.0	>9.4 bars	43-53°C	-	high threshold mechanothermal fibre	Georgopoulos (1976)
Monkey	hand	0.8±0.1	mean 5.95±0.59 bars	43.6±0.6°C	elliptical, 18.9±3.2mm²	mechanothermal nociceptive C-fibre afferent	LaMotte and Campbell (1978)
Monkey	hand	-	(i)QC mean 3.7 ±0.4 bars (ii)SC mean 7.3 ±2.4 bars	(i)44.0±0.4°C (ii)44.5±0.3°C	(i)28.4±4.7mm² (ii)34.5±5.9mm²	C-fibre nociceptive afferent	Meyer and Campbell (1981a)
Monkey	hand	0.81±0.05	5.35±0.44 bars	-	20.8±1.8mm²	CMH	Meyer and Campbell (1981b)
Monkey	hand	0.9±0.3	-	41 ->51°C (median 45°C)	-	CMH	LaMotte et al (1982)
Cat	foot	0.4-1.7	<300g./0.8mm²	40-55°C	-	C-heat receptor	Beck et al (1974)
Rat	tail	<2	14-400mN (median 52mN)	31-55°C	round or oval <25mm²	CMH	Fleischer et al (1983)

MECHANOTHERMAL NOCICEPTORS (mechanical heat and cold) - A fibre innervated

HAIRY SKIN

Species	Skin region	Conduction velocity of afferent fibre (m/sec)	STIMULUS THRESHOLD			Receptive field	Terminology used by authors	Authors
			mechanical	heat	cold			
Monkey	hand wrist foot,ankle	median 19.3	median 51mN	>51°C	not measured units responded to ice	-	mechanothermal nociceptor	LaMotte and Thalhammer (1982)

GLABROUS SKIN

Monkey	hand wrist foot,ankle	median 19.3	median 51mN	>51°C	not measured units responded to ice	-	mechanothermal nociceptor	LaMotte and Thalhammer (1982)
Monkey	hand	3-39	>9.4 bars	41-53°C	ice- 31°C	<5mm², 1-2 peaks of max. sens. or 9-225mm², 3 or more sens. spots	high threshold mechanothermal fibre	Georgopoulos (1976)

HAIRY SKIN

MECHANOTHERMAL NOCICEPTORS (mechanical heat and cold) - C fibre innervated

Species	Skin region	Conduction velocity of afferent fibre (m/sec)	STIMULUS THRESHOLD			Receptive field	Terminology used by authors	Authors
			mechanical	heat	cold			
Monkey	hand, wrist, foot,ankle	median 0.84	median 51mN	39-51°C	not measured units responded to ice	-	mechanothermal nociceptor	LaMotte and Thalhammer (1982)
Monkey	hand	mean 0.8±0.1	mean 3.33 bars	mean 43.6°C	not measured units responded to ice	mean 18.9±3.2mm	mechanothermal nociceptive C-fibre	LaMotte and Campbell (1978)
Cat	leg		>5g	55°C	-	-	-	Iggo (1959)
Rat	leg	0.5-0.8	not measured responded to pressure, pinch and prick.	>50°C	not measured responded to 8°C cooling	-	unspecific C fibres	Iriuchijima and Zotterman (1960)
Rat	tail	-	-	not measured 46°C only tested	not measured 4°C only tested	-	non-specific C fibres	Witt and Griffin (1962)

MECHANOTHERMAL NOCICEPTORS (mechanical heat and cold) - C fibre innervated

GLABROUS SKIN

Species	Skin region	Conduction velocity of afferent fibre (m/sec)	STIMULUS THRESHOLD			Receptive field	Terminology used by authors	Authors
			mechanical	heat	cold			
Monkey	hand	0.8-1.0	>9.4 bars	43-53°C	ice-25°C	<5mm², 1-2 peaks of max. sens., or 9-225mm², 3 or more sens. spots	high threshold mechanothermal fibre	Georgopoulos (1976)
Monkey	hand	mean 0.8±0.1	mean 5.95 bars	mean 43.6°C	not measured ice only tested	mean 18.9±3.2mm	mechanothermal nociceptive C-fibre	Lamotte and Campbell (1978)
Monkey	hand, wrist, foot, ankle	median 0.84	median 51mN	39-51°C	not measured ice only tested	-	mechanothermal nociceptor	LaMotte and Thalhammer (1982)

MECHANOTHERMAL NOCICEPTORS (mechanical and cold) - A fibre innervated

HAIRY SKIN

Species	Skin region	Conduction velocity of afferent fibre (m/sec.)	STIMULUS THRESHOLD		Receptive field	Terminology used by authors	Authors
			mechanical	cold			
Monkey	hand,wrist foot,ankle	median 19.3	median 51.0mN	not measured units responded to ice	-	mechanothermal nociceptor	LaMotte and Thalhammer (1982)

GLABROUS SKIN

Monkey	hand	3-39	>9.4 bars	31°C-ice	<5mm², 1 or 2 peaks of max. sensitivity, or 9-225mm² 3 or more sensitive spots	high threshold mechanothermal fibre	Georgopoulos (1976)
Monkey	hand,wrist foot,ankle	median 19.3	median 51.0mN	not measured units responded to ice	-	mechanothermal nociceptor	LaMotte and Thalhammer (1982)

MECHANOTHERMAL NOCICEPTORS (mechanical and cold) - C fibre innervated

HAIRY SKIN

Species	Skin region	Conduction velocity of afferent fibre (m/sec.)	STIMULUS THRESHOLD mechanical	STIMULUS THRESHOLD cold	Receptive field	Terminology used by authors	Authors
Monkey	hand	-	-	not measured units responded to ice	-	mechanothermal nociceptive C-fibre afferent	LaMotte and Campbell (1978)
Monkey	hand,wrist foot,ankle	median 0.84	median 51.0mN	not measured units responded to ice	-	mechanothermal nociceptor	LaMotte and Thalhammer (1982)
Cat	leg and foot	0.5-1.15	>5g.	<17°C	<5 x 5mm	cold receptor	Iggo (1959)
GLABROUS SKIN							
Monkey	hand	0.8-1.0	>9.4 bars	25°C-ice	<5mm², 1 or 2 peaks of max. sensitivity or 9-225mm² 3 or more sensitive spots	high threshold mechanothermal fibre	Georgopoulos (1976)
Monkey	hand	-	-	not measured units responded to ice	-	mechanothermal nociceptive C-fibre afferent	LaMotte and Campbell (1978)
Monkey	hand,wrist foot,ankle		Median 51.0mN	not measured units responded to ice	-	mechanothermal nociceptor	LaMotte and Thalhammer (1982)

THERMAL NOCICEPTORS (heat)- A fibre innervated

GLABROUS SKIN

Species	Skin region	Conduction velocity of afferent fibre (m/sec.)	STIMULUS THRESHOLD	Receptive field	Terminology used	Authors by authors
Monkey	hand	3-39	41-53°C	-	high threshold thermal fibre	Georgopoulos (1976)

THERMAL NOCICEPTORS (heat)- C fibre innervated

HAIRY SKIN

Cat	foot	0.4-1.7	40-55°C	-	C-heat receptor	Beck et al (1974)

GLABROUS SKIN

Monkey	hand	0.8-1.0	43-55°C	-	high threshold thermal fibre	Georgopoulos (1976)
Cat	foot	0.4-1.7	40-55°C	-	C-heat receptor	Beck et al (1974)

THERMAL NOCICEPTORS (cold)- A fibre innervated

HAIRY SKIN

Species	Skin region	Conduction velocity of afferent fibre (m/sec.)	STIMULUS THRESHOLD	Receptive field	Terminology used	Authors by authors
Monkey	hand, wrist ankle,foot	2.4-3.2	19-25°C	60-145mm²	high threshold cold receptor (HCR)	LaMotte and Thalhammer (1982)
GLABROUS SKIN						
Monkey	hand,wrist ankle,foot	6.4-9.8	23-26°C	4-12 mm²	HCR	LaMotte and Thalhammer (1982)
Cat	-	5.3	23°C	1mm²	HCR	LaMotte and Thalhammer (1982)

THERMAL NOCICEPTORS (cold)- C fibre innervated

HAIRY SKIN

Monkey	hand,wrist ankle,foot	0.9	19°C	36mm²	HCR	LaMotte and Thalhammer (1982)
GLABROUS SKIN						
Monkey	hand,wrist ankle,foot	0.87-1.10	not measured unit responded to ice	8-55mm²	HCR	LaMotte and Thalhammer (1982)

THERMAL NOCICEPTORS (heat and cold) - A fibre innervated

GLABROUS SKIN

Species	Skin region	Conduction velocity of afferent fibre (m/sec.)	STIMULUS THRESHOLD heat	cold	Receptive field	Terminology used by authors	Authors
Monkey	hand	-	>41°C	<31°C	-	high threshold thermal fibre	Georgopoulos (1976)

THERMAL NOCICEPTORS (heat and cold) - C fibre innervated

GLABROUS SKIN

Monkey	hand	0.8-1.0	43-53°C	25°C-ice	-	high threshold thermal fibre	Georgopoulos (1976)

Chapter 2.

CUTANEOUS NOCICEPTORS IN THE BEAK OF THE CHICKEN

2. CUTANEOUS NOCICEPTORS IN THE BEAK OF THE CHICKEN

I. INTRODUCTION

The aim of the experiments described in this chapter was to search for, and define the physiological characteristics of, cutaneous receptors which respond to noxious stimulation of the beak of the chicken.

Several experimental approaches were attempted. Initial experiments concentrated on microelectrode recording from the trigeminal ganglion, and single-unit dissection of primary afferent fibres from the ophthalmic nerve. Both of these preparations proved to be unsatisfactory, and were abandoned. The results described in this chapter were obtained from a preparation which was developed and found to be satisfactory: single unit dissection of primary afferent nerve fibres from the alveolar mandibular nerve which innervates the lower beak. Results obtained from the upper beak are not reported here, but they did not differ qualitatively from the results reported below.

The experimental noxious stimulus employed was heat. This stimulus modality was chosen for several reasons. Firstly, during the process of beak trimming, the heat radiating from the debeaker blade as it approaches the beak must affect any

heat-sensitive receptors present in the beak before the blade makes mechanical contact with it. Secondly, heat is an easily controlled and reproducible stimulus. Thirdly, heat has been used extensively by previous investigators to define the characteristics of nociceptors in different species, making it possible to compare the present results with those of other investigators.

II. METHODS

Animals

The experiments were performed on adult Brown Leghorn hens. The birds were hatched and reared at the A.F.R.C. Poultry Research Centre, Roslin. They were individually housed in cages and had free access to food and water. At the time of the experiments the birds were 4 to 7 months old and they weighed 0.7 to 1.7 kg.

Anaesthesia

The birds were anaesthetized with urethane (ethyl carbamate), 1.5g./Kg. body weight, injected as a 25% solution in saline into the brachial vein. Following the procedure advocated by King and Biggs (1957), anaesthesia was induced in two stages. The first stage consisted of an initial rapid injection of one-third of the calculated dose to produce light anaesthesia, sufficient to control the bird. This was followed by a slow injection over a period of 10 to 15 minutes until the comb-pinch reflex was abolished. The brachial vein was then cannulated for subsequent administration of anaesthetic as required during the experiment.

The surgical preparation

A tracheotomy was performed and a tracheal cannula was inserted. This procedure facilitated the removal, using a cotton wool bud, of tracheal muc us which accumulated and occasionally caused respiratory distress during the course of an experiment.

The core temperature of the bird was maintained at 40 - 42°C by wrapping the bird in the blanket of a homoeothermic temperature control system (Bioscience, model CFP 8185). Feedback control of the temperature was provided by a thermister probe inserted into the rectum of the bird.

The skin overlying the cranium was incised and reflected, the periosteum was removed and the bone cleaned. Three holes, 1mm diameter, were drilled into the bone using a dental drill. A specially designed and built head holder was attached to the cranium with three self tapping screws and dental acrylic cement (Simplex Rapid, Howmedica). This head holder consisted of a stainless steel plate (20 x 25 x 0.5 mm.) shaped to the cranium contour, welded to a stainless steel rod with its long axis perpendicular to the plate. This rod was firmly clamped in a horizontal position to a pillar screwed into a steel experimental table (Clark and Ramsay, 1975). The upper and lower beak were held in an open position by cementing (dental

acrylic cement) their right hand sides to a cork pad attached to a brass block. This block was firmly clamped to the experimental table. This arrangement held the skull and beak rigid, in a horizontal position with the left side uppermost.

The mandibular skin was incised and reflected. The alveolar mandibular nerve (fig. 1:4) was then visible through the thin layer of bone overlying the mandibular canal. This bone was carefully removed, using a curette and fine forceps, exposing the nerve in the mandibular canal. The operating area was illuminated by a fibre optic light source (Barr and Stroud) and viewed through a stereoscopic dissecting microscope (Nikon). The nerve was carefully separated from the accompanying blood vessels and connective tissue. A dissecting pool was constructed by suturing the edges of the mandibular skin flaps to a stainless steel ring which was rigidly clamped to the experimental table.

The nerve was carefully lifted onto a small black Perspex platform, which was rigidly mounted on a micromanipulator fixed to the experimental table. The nerve sheath was slit open and carefully peeled off. With the aid of specially sharpened watchmaker's forceps, fine razor blades and insect pins, small nerve bundles were dissected from the main trunk. Each bundle was further dissected into fine filaments on the Perspex platform (Paintal, 1953; Iggo, 1955, 1957).

Electrical Recording

Electrical activity was recorded by carefully lifting a filament onto one pole of a bipolar, silver wire, hook electrode. The other pole was connected to a small strand of the nerve sheath, the sheath being connected to earth via the animal and table. The recording electrode was rigidly mounted in a micromanipulator. Electrical activity was amplified with an A.C. differential preamplifier (W.P. Instruments, DAM 5A or 6A) using a bandwidth of 100 Hz to 10 kHz. The electrical activity was monitored on a dual-beam storage oscilloscope (Tektronix, 5103N) and an audio amplifier and loudspeaker. The activity was stored on one channel of a four channel FM tape recorder (Racal, Store 4DS), at a tape speed of 15/16 ips.

The nerve filament was dissected further, by successive longitudinal splitting, to the point where the electrical activity recorded consisted of a single active unit or not more than three easily distinguished units. Electrical activity was defined as a single unit on the basis of the consistent amplitude and duration of the action potential when observed on the oscilloscope using a fast sweep speed, triggering the oscilloscope from the preceding action potential. A signal-to-noise ratio of at least 3:1 was found to be necessary for the unambiguous identification of a single unit using this

technique. In an ideal situation, identification of a single unit can be verified by the collision technique (Iggo, 1958). This was not feasible in the present experiments because the small size of the dissecting pool and the short length of nerve trunk available (8 to 10 mm.) made it impossible to position stimulating electrodes satisfactorily.

Stimulation

A. Electrical

Direct electrical stimulation of the nerve trunk, in order to measure the conduction velocity of the axon innervating the receptor under study, was not possible for the reasons given above. The alternative approach adopted was to attempt to electrically stimulate the axon close to thereceptive field of the receptor (Gottschaldt, Iggo and Young, 1973). It was not possible to stimul ate axons with the poles of the stimulating electrode positioned on the surface of the beak, presumably due to the insulating properties of the horn overlying the epidermis. This problem was overcome by piercing two small holes in the horn, one on either side ofthe RF of the receptor. The poles of the bipolar stimulating electrode, mounted on a micromanipulator, were then carefully inserted into the two holes. To prevent recording from receptors which may have been damaged or sensitized by the electrode insertion procedure, the conduction velocity measurements were made at the end of the

recording session prior to killing the bird. This constraint on the use of the procedure resulted in a limited number of conduction velocity measurements being made.

Electrical stimuli were delivered through an isolated stimulator (Digitimer, DS2), triggered by a quartz clock (Digitimer, D100). Conduction distances were measured by placing a thin cotton thread along the path of the nerve from the recording electrode to the RF, and then measuring the thread. Conduction velocites were calculated from the conduction time and conduction distance.

B. Mechanical

Mechanical stimulation of the beak surface was carried out with a variety of paintbrushes, glass and wooden probes, and a set of von Frey hairs. The probes had rounded ends, with tip diameters of 0.5 to 2mm. They were hand-held or rigidly mounted in a micromanipulator. The von Frey hairs exerted forces of 0.3 to 325g.. They were calibrated using an electronic balance.

C. Thermal

An attempt was made to construct a suitable contact thermode to enable controlled thermal stimuli to be delivered to the beak

surface. This approach was abandoned as the device proved to be unsatisfactory in practise, major problems being precise control of stimulus parameters and the simultaneous mechanical stimulation of the RF caused by the thermode.

1. Heat

A satisfactory means of heating the beak surface was found by building a radiant-heat stimulator, based on that described by Beck et al (1974). This device enabled the surface of the beak to be maintained at a constant preset temperature for a preset time period. The heating element consisted of a prefocussed projector bulb with a built in reflector (12V, 100W, Atlas A1/231). The bulb was oriented vertical to the beak surface. Temperature was measured with a thermocouple (copper-constantan, 0.5mm. long tip) placed in firm contact with the surface of the beak, in the centre of the bulb's focus (focal length 35mm, focus 10mm diameter). The output voltage from the thermocouple was compared with a preset control signal, the heating current to the bulb being automatically adjusted to the control level. Heating parameters controlled by the stimulator were rate of temperature rise (0.5 to 20 °C/sec.) final temperature level or plateau (35°C to 65°C) and the duration of the temperature plateau (0 to 30 sec.). Accuracy of the temperature control was ±0. 5 °C. The thermocouple output was calibrated against two mercury-in-glass

thermometers and a platinum resistance thermometer (Advance 9035NN 01AX, accuracy 0.1°C), and it was linear over the range 0-65 °C). Calibration was checked before each experiment.

The stimulator was triggered by the Digitimer D100. The output of the thermocouple was displayed on the oscilloscope screen and recorded on the second channel of the tape recorder. The third channel of the tape recorder was used for flutter compensation, i.e. to reduce any extraneous noise in the D.C. temperature recording caused by small fluctuations in tape speed. The fourth channel was used to record a voice commentary during the experiment.

2. Cold

The beak surface was cooled by positioning a small test tube filled with iced water above the beak surface. The test tube was held either manually or mounted in a micromanipulator. The beak surface temperature was measured with the thermocouple of the radiant heat stimulator. This relatively simple procedure, provided a reliable method of testing for responsiveness to cooling.

Small chips of ice, held in forceps were used to locate RF's.

Experimental procedure

Receptors were identified by the following procedure.

A single nerve filament was placed onto the recording electrode. Cutaneous search stimuli were applied to the surface of the beak to determine if the filament contained any active units. When a RF was located, the filament was further dissected to the point where the impulse activity consisted of a single unit. Search stimuli were applied as follows. The surface of the beak was mechanically stimulated using firstly a paintbrush, then glass and wooden probes, i.e. light touch and pressure. If a RF was found, the mechanical threshold was determined by using the von Frey hairs in ascending order of magnitude. The RF was mapped out using the threshold value von Frey hair, marking the surface of the beak using a black fibre tip pen (tip diameter 0.5 mm). The RF was recorded on a scale diagram of the beak.

If no RF was located, cold search stimuli were used. The iced water-filled test tube was applied directly to or held over the surface of the beak. Small chips of ice held in forceps were used to stimulate the internal surface of the beak and to more precisely determine the location of the RF. Once a RF had been located, either with mechanical or cold stimulation, the thermocouple was placed in contact with the beak in the

geometric centre of the RF. Cold sensitivity was tested by positioning the iced water-filled test tube over the centre of the RF, approximately 1 cm. away from the beak surface. The temperature of the RF could then be lowered by bringing the test tube closer to the beak surface.

The unit under study was then tested for heat sensitivity using the radiant-heat simulator. The RF was heated to a baseline temperature of 34 °C, then the temperature was raised, at a rate of 1 °C/sec, to 60 °C. If the unit responded at any point during the temperature ramp, the ramp was terminated and a note taken of the temperature at which the unit fired.

Stimulus-response relationships of heat-sensitive receptors

A detailed study was made of heat-sensitive receptors using the following procedure.

A ramp-and-hold heat stimulus profile was used. The baseline temperature was 34 °C, the rate of temperature rise was 1 °C/sec., and the hold duration was 10 sec. (see fig.2:2). Heat threshold was determined by delivering a stimulus with a hold temperature equivalent to that at which the unit first responded during the test heat stimulus. If the unit responded at this temperature, a further stimulus was delivered with a hold temperature 1 °C lower. If the unit did not respond, a stimulus

with a hold temperature 1 °C higher was delivered. This procedure gave a measure of the threshold to the nearest 1°C. Heat stimulus-response curves were obtained by repeated stimulation at different suprathreshold temperatures, at 2 °C intervals. Stimuli were randomized using random number tables. The time interval between stimuli was 3 minutes (Beck et al, 1974) in order to avoid any sensitizing or depressing effects of epidermal damage caused by faster repetition rates (Beck et al, 1974; LaMotte and Campbell, 1978). The variability of the stimulus response relationship was tested by repeated stimulation at each temperature.

Data analysis

Permanent records of data were obtained by replaying the tape-recorded material onto the oscilloscope and photographing the screen with a Polaroid camera, and by replay onto an ultraviolet oscillograph (Bell and Howell, S-127). Measurements of "instantaneous" frequency (the reciprocal of the interspike interval) were made using a ratemeter (Neurolog system module NL255, Digitimer Ltd.).

Heat stimulus-response curves

The procedure of Beck et al (1974) was employed for the construction of heat stimulus-response curves. The number of

impulses discharged during each 10 sec. hold temperature was plotted against the RF temperature. Impulses were counted manually from the UV paper trace, using a paper speed sufficient to clearly distinguish each impulse in the spike train.

Statistical Procedures

The mathematical description of the shape of a stimulus-response curve is normally termed the "intensity function". Three candidate intensity functions were considered for their possible goodness-of-fit to the stimulus-response curves obtained in the present experiments. These functions and their respective equations are:

(a) Linear , $R=b.S + A$

(b) Logarithmic, $R = b.\log S + A$

(c) Power, $R = A.S^b$

where R= response magnitude

b=slope

S=stimulus intensity

A=intercept

A stimulus-response relationship is approximated by a particular function if a straight line relationship is obtained

when, respectively, R is plotted against S for a linear function, when R is plotted against log S for a logarithmic function, or when log R is plotted against log S for a power function.

A quantitative approach to determining the goodness-of-fit for each intensity function to each stimulus-response curve was undertaken by performing linear regression analysis on the stimulus-response data, both untransformed and after the logarithmic transformations. In order to avoid the distorting effects of different thresholds and ranges on log-transformed values, stimulus values were normalized. Normalization was carried out with respect to threshold by allocating the threshold temperature, Tt, the value of 1, thus Tt=1, Tt+2 =3, Tt+4 =5, etc. This normalization procedure corresponds to that employed by Georgopoulos (1977), making feasible a direct comparison of the present data with his results. Applying this normalization technique, the formulae describing the three intensity functions become:

(a) Linear , $R=b.(S-S_o) + A$

(b) Logarithmic, $R = b.\log (S-S_o) + A$

(c) Power, $R = A.(S-S_o)^b$

where S_o = highest subthreshold intensity, i.e. 1 °C below threshold.

The correlation coefficient, r, was used as the index of goodness-of-fit of each stimulus-response curve to each intensity function. Another approach used was to determine the best fitting function for the pooled data, i.e. the population intensity function, rather than for individual units (Georgopoulos, 1977). The best fitting population intensity function was determined by pooling the correlation coefficients for each function. Georgopoulos (1977) employed the coefficient of determination, r^2, as the measure of goodness-of-fit of his data to a particular function. This statistic is the equivalent of r in multiple regression analysis. Its use in this context is unusual but statistically legitimate (Waddington, D., pers. comm. 1984). Values of r^2 for the present data were calculated for comparison with the results of Georgopoulos (1977).

Linear regression analysis was carried out using the method of least squares using a laboratory statistical package on a minicomputer (MINC PDP 11/23). Mann-Whitney U tests (Siegel, 1956) were used to test the significance of differences in means of the samples. Unlike the Student's t-test this test does not assume normal distributions of the variables. Tests were performed using an electronic calculator or by using a statistical package (MINITAB) on the Poultry Research Centre PRIME computer.

III. RESULTS

A total of 171 single units was recorded from fine filaments dissected from the alveolar mandibular nerve from 30 birds. The units were divided into three main functional groups on the basis of their responses to the three modalities of cutaneous stimulation, mechanoreceptors, cold receptors and nociceptors. This chapter will concentrate on the properties of the nociceptors. A brief summary of the distinguishing features of the mechanoreceptors and cold receptors is included here. It must be emphasized that the numbers of the different receptors recorded probably do not represent the true proportions of the different receptor types present in the lower beak. This is because once the identifying characteristics of each receptor type had been established, a deliberate attempt was made to search for nociceptors, and numerous nerve filaments containing only mechanoreceptors and cold receptors were discarded without further investigation.

MECHANORECEPTORS

108 units were recorded which responded only to mechanical stimulation of the beak surface. The units could be divided into two groups on the basis of their response to a sustained mechanical indentation of the receptive field, rapidly and slowly adapting mechanoreceptors.

Rapidly adapting mechanoreceptors

96 units were classified as rapidly adapting mechanoreceptors. None of the units carried a resting discharge. The units responded only to a moving mechanical stimulus. A sustained mechanical indentation of the beak surface produced a discharge during the onset of the stimulus. The discharge ceased during the sustained, static, part of the stimulus.

The rapidly adapting mechanoreceptors could be subdivided into two groups on the basis of their response to a moving mechanical stimulus. Type (i) responded with a continuous train of impulses during the movement phase of the stimulus. Type (ii) responded with only a few impulses (1 - 3), discharged at the onset and sometimes the removal of such a stimulus. Mechanical force thresholds (measured with von Frey hairs) ranged from 4 to 50g for type (i) and 1.4 to 50g for type (ii).

Neither type (i) nor type (ii) responded to cold (down to 0°C) or heat (up to 60°C)stimulation of the receptive field.

Receptive fields were circular or roughly elliptical in shape, with the longest diameter of each receptive field ranging from 0.5 to 9mm. for type (i) and 0.5 to 17mm for type (ii). The

receptive fields for both types were distributed evenly over the beak surface, down to the tip of the beak.

Conduction velocities for type (i) units ranged from 30.7 to 54.3 m/sec (Mean 43.6±4.9, median 46.8, n=4), and for type (ii) units ranged from 21.25 to 50.5 m/sec (mean 38.1±5.8, median 41.9, n=5).

Slowly adapting mechanoreceptors

12 Units were classified as slowly adapting mechanoreceptors. None of these units carried a resting discharge. A sustained suprathreshold mechanical indentation of the beak surface produced a continuous discharge which lasted for the duration of the stimulus. Mechanical force thresholds ranged from 12 to 125g. There was no response to cold (down to 0°C)or to heat (up to 60°C)stimulation of the receptive field.

Receptive fields were circular or elliptical in shape, the longest diameter of each receptive field ranging from 1 to 2 mm. The receptive fields were distributed evenly over the beak surface, down to the beak tip.

Conduction velocities of the slowly adapting mechanoreceptors ranged from 0.65 to 1.98 m/sec. (mean= 1.49±0.3, median 1.92, n=4).

COLD RECEPTORS

27 units were classified as cold receptors. These units carried a spontaneous discharge in the absence of any experimental stimulation. This discharge was regular in pattern. The discharge frequency was temperature dependent. A dynamic and static response to cooling the beak surface was observed. A sustained cooling of the receptive field produced an initial increase in impulse frequency. This frequency declined to a lower level which was sustained for the durat-ion of the stimulus. Rewarming of the receptive field back to the prestimulus temperature produced a transient cessation of discharge. The discharge reappeared and returned to the prestimulus frequency. A sustained warming of the receptive field above 36 °C (up to 60 °C)produced a cessation of the discharge. Recooling the receptive field back to the original prestimulus temperature resulted in the reappearance of the discharge.

The discharge of the units was not altered by mechanical stimulation of the receptive field, ruling out the possibility that these units may have been thermally sensitive mechanoreceptors.

Receptive fields for these units were roughly circular in

shape. It was not possible to measure them accurately, but the longest diameter never exceeded 3mm. The receptive fields were distributed evenly over the beak surface, down to the beak tip.

NOCICEPTORS

Thirty-six units were classified as nociceptors. They had the following general characteristics: none of these units carried a resting discharge; all thirty-six units responded to heating the receptive field with threshold above 40 °C; thirty of the units also responded to mechanical stimulation of the receptive field. None of the units responded to cooling the receptive field (down to 0 °C). A detailed account of the heat stimulus-response characteristics of these units now follows.

1. The response of the nociceptors to heat stimulation

(i) Heat thresholds

The distribution of the heat thresholds for all 36 units is illustrated in fig.2:1. The thresholds ranged from 41 to 56 °C with a mean value of 45.9±0.66 °C (S.E.) and a median value of 46 °C.

(ii) Stimulus response curves: general features

A typical example of the response of a nociceptor to heating is illustrated in fig.2:2A. This unit had a receptive field

located 3mm proximal to the tip of the beak. Its mechanical threshold was 50g., and its heat threshold was 46 °C. Increasing the stimulus intensity above threshold produced an increased number of impulses discharged. The discharge pattern was continuous and irregular; the discharge frequency appeared to increase then decrease during each stimulus. The discharge did not outlast the stimulus period. A discontinuous (bursting) discharge during heat stimulation was noted for 2 units (5.56% of sample). The accompanying graph (fig.2:2B) illustrates the response of this unit, measured as the number of impulses discharged per 10 sec. stimulus plateau plotted against the receptive field temperature. The response increased in a non-linear fashion with increasing temperature, resulting in a sigmoid stimulus-response curve. This curve reached a peak at 54°C, the response at 56 °C being slightly lower.

The stimulus-response data for all 36 units is presented in table 2:1 and is illustrated graphically in fig 2:3. All the units displayed an increasing response with increasing temperature. There is variability in the response characteristics in terms of threshold temperature, response magnitude, shape and slope of the curves, and the range of temperatures over which the units responded. Twenty five units (69.4% of the sample) showed a peak response, increasing the temperature beyond this peak produced a decrease in the

response. The remaining 11 units displayed an increasing response up to the highest temperature tested. For these 11 units, the response at the highest temperature tested will be referred to as the maximal response.

The heat threshold, the temperature for the peak or maximal response, the temperature range from threshold to peak/maximal response, and the amplitude of the peak/maximal response for all 36 units are presented in tables 2:2 and 2:3 and summarized in table 2:4.

(iii) Peak/ maximal temperature

The distribution of the temperatures at which the 36 units showed a peak/maximal response is illustrated in fig. 2:4. Of the 25 units which displayed a peak response, 18 peaked at temperatures between 47 and 54°C. The remaining 7 of these 25 units peaked between 56 and 60°C; of these, 6 had very low amplitude responses (between 9 to 15 impulses per 10 sec.).

Of the 11 units which displayed an increase in discharge up to the highest temperature tested, 9 were tested up to 51 to 54°C, and two up to 56°C.

(iv) Peak/maximal responses

The distribution of the peak/maximal responses for the 36 units is illustrated in fig. 2:5. The 25 peak responses ranged from 9 to 191 impulses per 10 sec. The median value was 59 impulses per 10 sec., with just over one half of the units (n=13) showing a peak response between 40 to 80 impulses per 10 sec. Three groups can be distinguished, with peaks of 0 to 20, 40 to 120, and 140 to 200 impulses per 10 sec respectively.

The maximal responses of the 11 units which did not display a peak response within the temperature range tested ranged from 74 to 224 impulses per 10 sec., with a median value of 101 impulses per 10 sec. Over half the units (7) had maximal responses of 80 to 120 impulses per 10 sec.

The relationship between the peak/maximal response and threshold temperature, and between peak/maximal response and peak/maximal temperature is illustrated in fig. 2:6. The group of units with very low amplitude responses had high thresholds (48 to 56 °C) and high peak/maximal temperatures (56 to 60°C). For the rest of the units there was no apparent correlation between the peak/maximal response and threshold and between peak/maximal response and peak/maximal temperature.

(v) Thermal range

The relationship between threshold temperature and peak/maximal temperature is illustrated in Fig. 2:7. A positive trend seems to hold for this relationship, the units with the lowest peak/maximal temperatures having the lowest threshold temperatures and units with the highest peak/maximal temperatures having the highest threshold temperatures. The thermal range of a unit is here defined as the temperature range from threshold to peak/maximal response. The thermal ranges for the 25 units showing a peak response, i.e. threshold temperature to peak temperature (Tp-Tt), varied from 2 to 12°C, median of 6°C and mean of 6.24± 0.48°C (S.E.). Just under half the units (10) had the median value of 6°C.

The thermal ranges for the 11 units which showed an increased response up to the highest temperature tested, i.e. threshold temperature to highest temperature tested (Th-Tt), varied from 4 to 14°C, median of 6°C and mean of 8.00 +0.94°C(S.E.).

The thermal ranges of all 36 units are also illustrated in Fig. 2:8. Here the stimulus-response curves are plotted out with temperatures normalized with respect to the heat threshold. For convenience of illustration, the stimulus-response curves are divided into the same 5 groups as in fig. 2:3.

(vi) Repeatability of stimulus-response curves

The 36 stimulus-response curves illustrated in fig.2:3 are, in most cases non linear. Some of the curves can be described as positively accelerating (i.e. concave upwards), some as negatively accelerating (i.e. convex upwards) and some as a combination of these two shapes (i.e. sigmoid). To assess the biological variation in the stimulus-response curves, i.e. the repeatability of the observations, three of the units were tested several times as described in the methods section. The results are illustrated in fig.2:9. The shape of each curve remained relatively constant from trial to trial, and no pattern could be detected in the change in responsiveness from trial to trial.

(vii) Stimulus-response coding

A visual impression of goodness of fit for each of the three intensity functions tested can be gained from fig.2:10. Here the data from unit 36 has been subjected to logarithmic and power transformations and plotted on linear axes to compare with the untransformed data. In each case the mean value of three observations for each temperature tested was used. The untransformed stimulus-response curve is curvilinear and postively accelerating. Logarithmic transformation of the stimulus increases the curvature, and logarithmic

transformation of both stimulus and response substantially straightens out the curve.

The effect of normalizing the stimulus values and subsequently subjecting the data to logarithmic and power transformations is illustrated in fig.2:11. The logarithmic transformation extends the lower end of the curve and compresses the upper end, transformation of the stimulus alone produces a more pronounced curvature, whilst transformation of both stimulus and response results in a close approximation to a straight line. Figure.2:12. shows the calculated regression line fitted to the data of fig.2:11. The correlation coefficients are 0.8791 for the untransformed data, 0.7183 for the stimulus-transformed data, and 0.9938 for the stimulus- and response- transformed data. The correlation coefficient for the logarithmic function is not significantly different from zero ($P>0.05$); the values for the linear and power functions are significant at the $P<0.05$ and $P<0.001$ levels respectively. Regression analysis was carried out on the data from units 26 and 34 after normalization and transformation as described above. The correlation coefficients are given in table 2:5. Again the correlation was highest for the power function, the next best fit being the linear function.

The stimulus-response data for the 36 single-trial curves (including the first stimulus trial for units 26, 24 and 36)

were subjected to the analysis described above, to determine which function best fits the population of units. In each case the portion of the curve from threshold to peak (or threshold to the highest temperature tested) was used. The summary statistics of these calculations are presented in tables 2:6 and 2:7. Out of the 25 units tested to peak response 2 units (8%) were equally well fitted by all three functions. These units were 22 and 23, both very low-response units, with only two data points from threshold to peak hence the correlation coefficient in both cases for all three functions is 1.000. Two units, (8%), 28 and 30, showed a poor correlation ($p>0.05$) with all three functions. Of the remaining 21 units, seven (28%) were best fitted by the linear function, one (4%) by the logarithmic function and thirteen (52%) by the power function.

Of the eleven units not tested to peak response ten (91%) were best fitted by the power function and one (9%) by the logarithmic function.

Pooling the data for all 36 units, the number of units which were best fitted by a particular function are:

linear	7	(19.4%)
logarithmic	2	(5.6%)
power	23	(64.0%)

Two units (5.6%) were equally well fitted by all three functions and 2 (5.6%) units showed a poor correlation with

all three functions.

The best fitting function for the data as a whole, i.e. the population intensity function, was determined by pooling the correlation coefficients of all 36 units for each of the three functions (see table 2:8). Using this approach, the sample of units tested to peak response (n=25) was best fitted by the linear function, the sample of units not tested to peak response (n=11) was best fitted by the power function, as was the population of units as a whole (n=36).

(viii) Sensitivity

The exponent, b, of the power function, i.e. the slope of the fitted regression line was calculated for all 36 units. The values are given in tables 2:6 and 2:7. They ranged from 0.51 to 2.28 with a median of 1.59 and mean of 1.48 ± 0.09(SE) for the 25 units tested to peak response. For the data as a whole, i.e. adding on the 11 units not tested to peak response, the values are shifted upwards, ranging from 0.51 to 2.38 with a median of 1.63 and a mean of 1.58 ± 0.07(SE). The distribution of the slope (sensitivity) values is illustrated in fig2:13.

2. Response to mechanical stimulation

Thirty of the 36 nociceptors also responded to mechanical

stimulation of the receptive field. Mechanical force thresholds ranged from 1.4 to 125g, median 25g. A persistent discharge was evoked during sustained suprathreshold mechanical stimulation of the receptive fields.

3. Receptive fields

Receptive fields were circular or elliptical in shape, longest diameters ranging from 1 to 5 mm. They were distributed evenly over the beak surface, down to the beak tip.

4. Conduction velocities

Conduction velocities of the afferent fibres innervating the nociceptors ranged from 0.53 to 1.85 m/sec (mean 1.20 ± 0.15, median 1.14, n=9).

Fig. 2:1. Distribution of the heat thresholds of 36 heat-sensitive nociceptors recorded from the beaks of normal birds. Each threshold was measured to the nearest 1°C. Range =41 to 56°C, median 46°C, mean 45.9 ±0.66°C (S.E.).

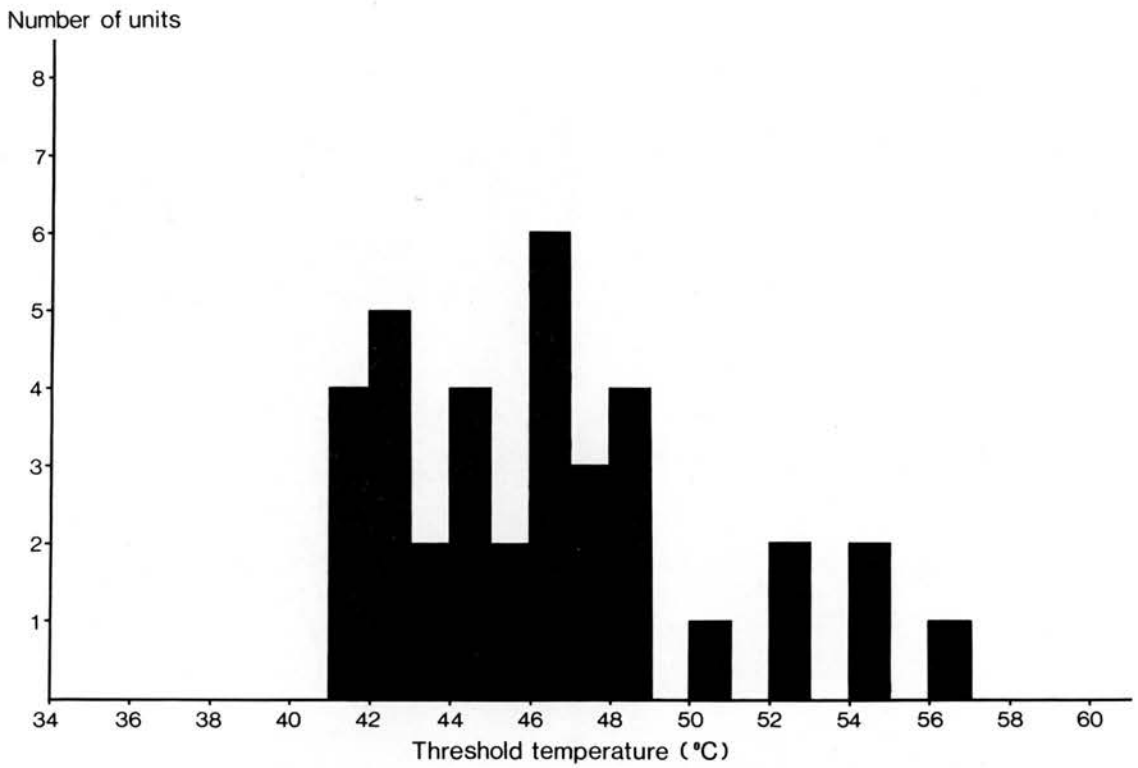

Number of units
8
7
6
5
4
3
2
1
34
36
38
40
42
44
46
48
50
52
54
56
58
60
Threshold temperature (°C)

Fig 2:2. The afferent discharges of a cutaneous nociceptor recorded from the alveolar mandibular nerve of an anaesthetized domestic hen, at different beak temperatures.

A. Specimen records. In each record, the lower trace is the action potential discharge and the upper trace is the beak surface temperature measured with a thermocouple positioned on the centre of the receptive field. The temperature of the receptive field was first raised to 34°C, then ramp and hold heat stimuli at different temperatures were delivered. The stimuli were applied in random sequence, with 3 minutes between successive stimuli. The hold temperature of each stimulus was maintained for 10 sec. The value of the hold temperature is indicated on the left of each record.

Note the increased discharge with increasing temperature. Unit no. 8. The receptive field of this unit was located 3mm. proximal to the tip of the beak. The receptive field was elliptical, 1 x 3 mm., oriented with its long axis parallel to the long axis of the beak. Its mechanical threshold, measured with von Frey hairs, was 50 g.

Calibration bar (time) = 10 sec.

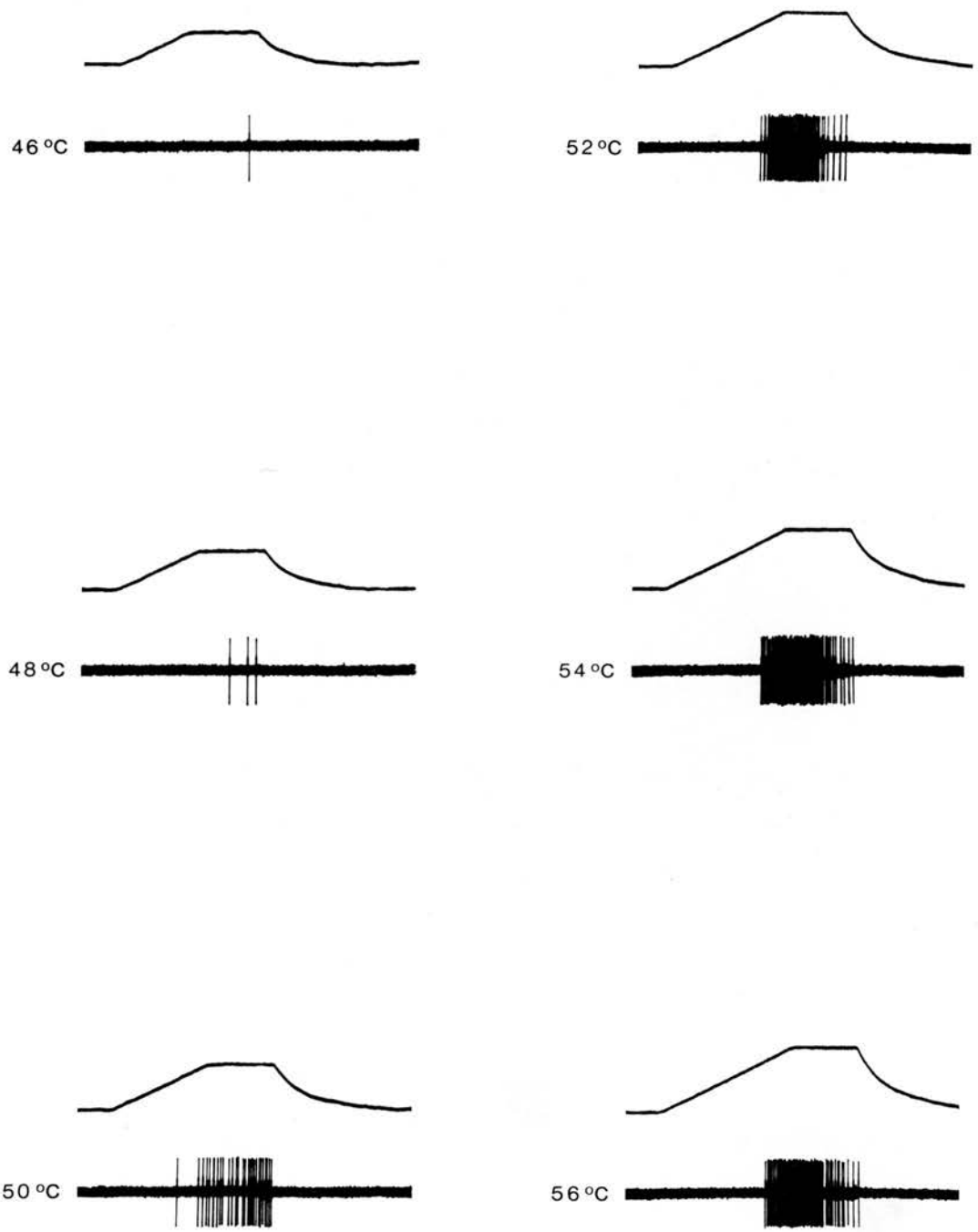

46 °C
52 °C
48 °C
54 °C
50 °C
56 °C

Fig. 2:2. The afferent discharges of a cutaneous nociceptor recorded from the alveolar mandibular nerve of an anaesthetized domestic hen, at different beak temperatures.

B. The relationship between the receptive field hold temperature and the response of unit no. 8, measured as the number of impulses discharged during the 10 sec. hold. Note the non-linearity of the stimulus-response relationship, and the peak response at 54°C.

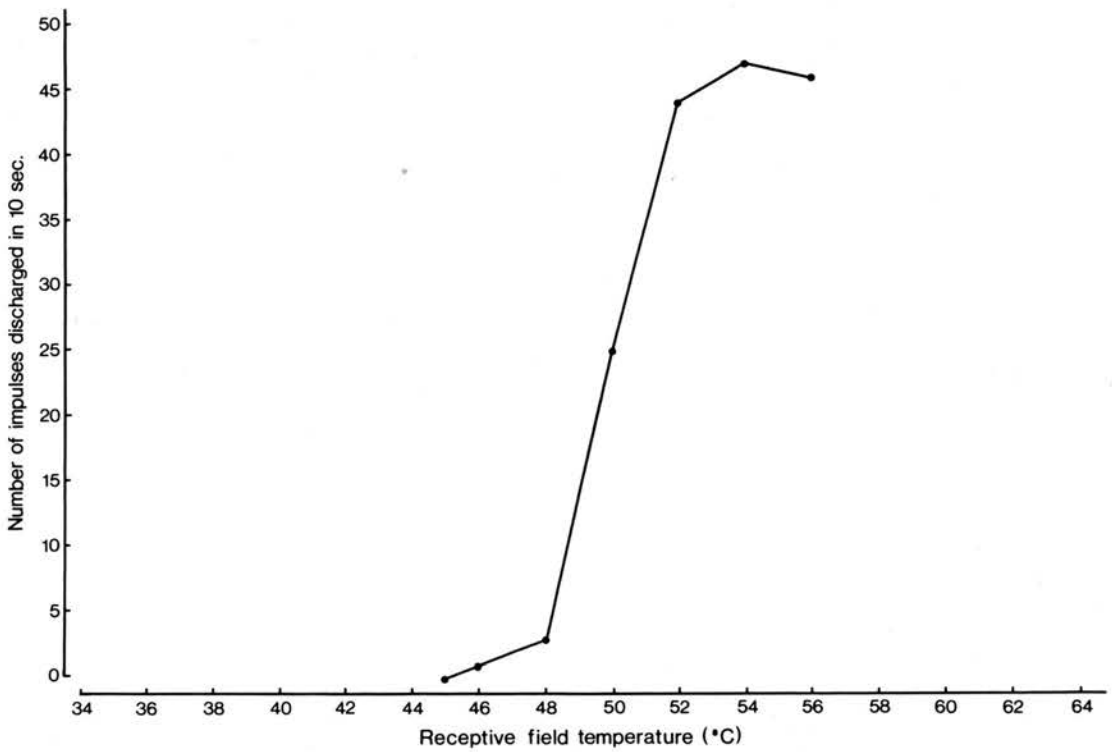
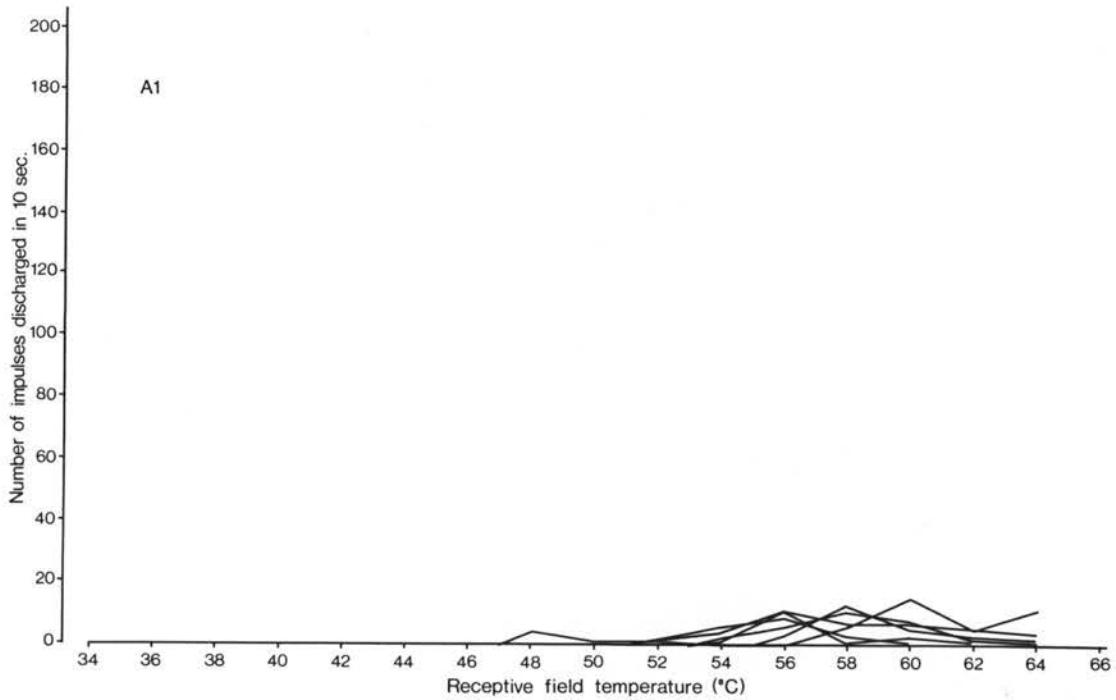
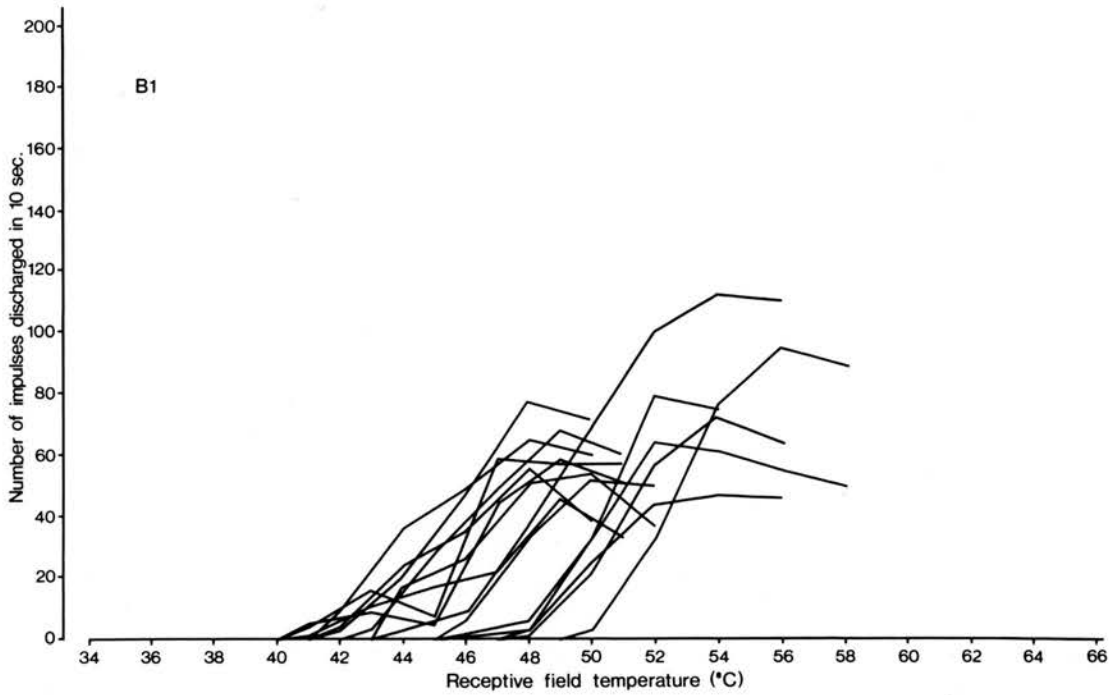
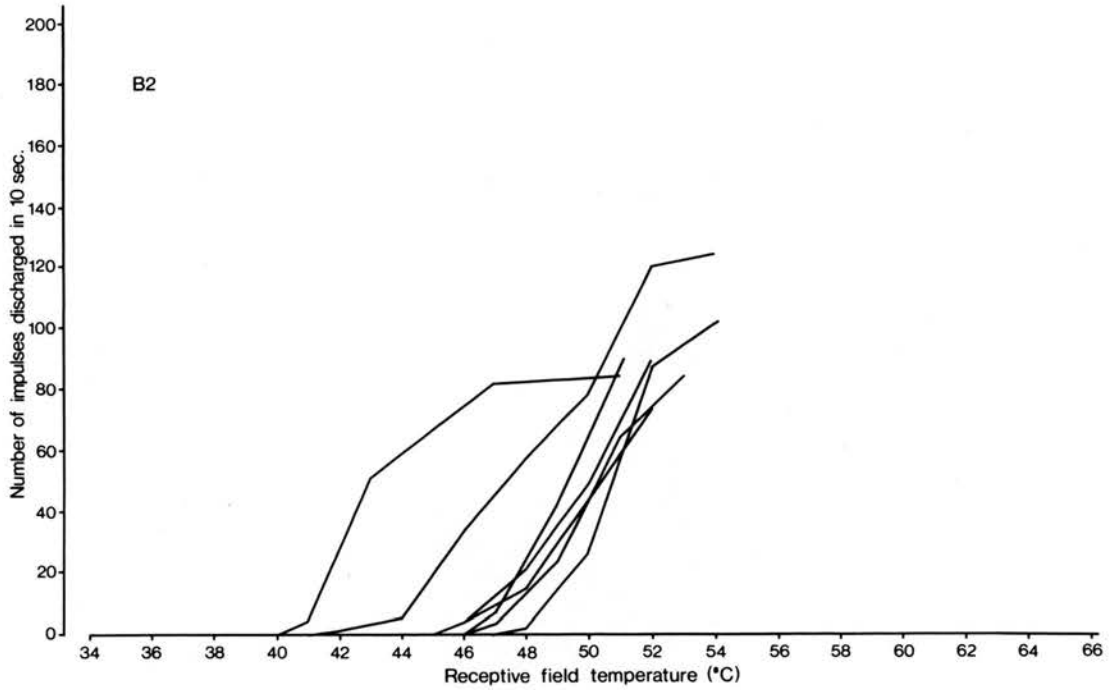
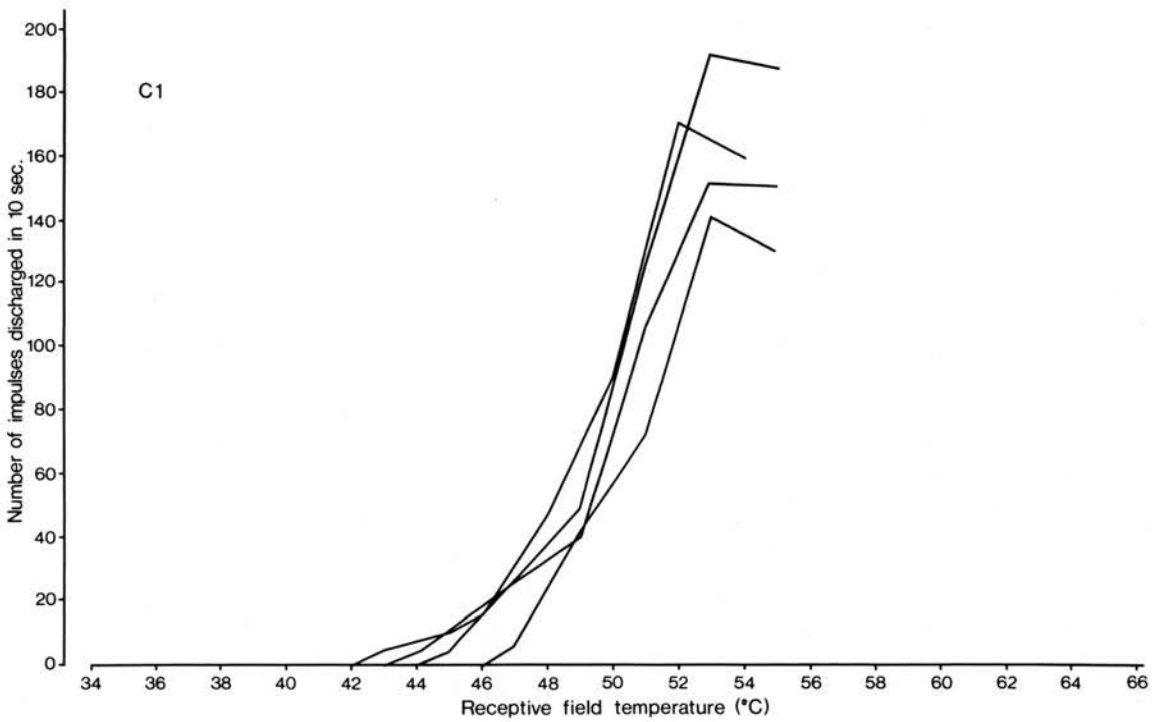

Number of impulses discharged in 10 sec.
50
45
40
35
30
25
20
15
10
5
0
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
Receptive field temperature (°C)

Fig. 2:3 The stimulus-response curves for all 36 heat-sensitive nociceptors recorded from the beaks of normal birds. For convenience of illustration they are presented in five groups. The criterion by which they are grouped is the value of the peak or maximal discharge. Groups A1, B1 and C1 contain the units which had peak discharges of <20 impulses per 10 sec (n=6), 40 to 130 impulses per 10 sec (n=15) and >140 impulses per 10 sec (n=4), respectively. Groups B2 and C2 contain the units which did not show a peak discharge within the range of temperatures tested. These units are grouped according to the maximal discharge observed, thus units with maximal discharges of 20 to 130 impulses per 10 sec. (n= 7) and >140 impulses per 10 sec (n= 4) are contained in B2 and C2 respectively.


A1
Number of impulses discharged in 10 sec.
200
180
160
140
120
100
80
60
40
20
0
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
66
Receptive field temperature (°C)


B1
Number of impulses discharged in 10 sec.
0
20
40
60
80
100
120
140
160
180
200
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
66
Receptive field temperature (°C)


B2
Number of impulses discharged in 10 sec.
200
180
160
140
120
100
80
60
40
20
0
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
66
Receptive field temperature (°C)


C1
Number of impulses discharged in 10 sec.
0
20
40
60
80
100
120
140
160
180
200
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
66
Receptive field temperature (°C)

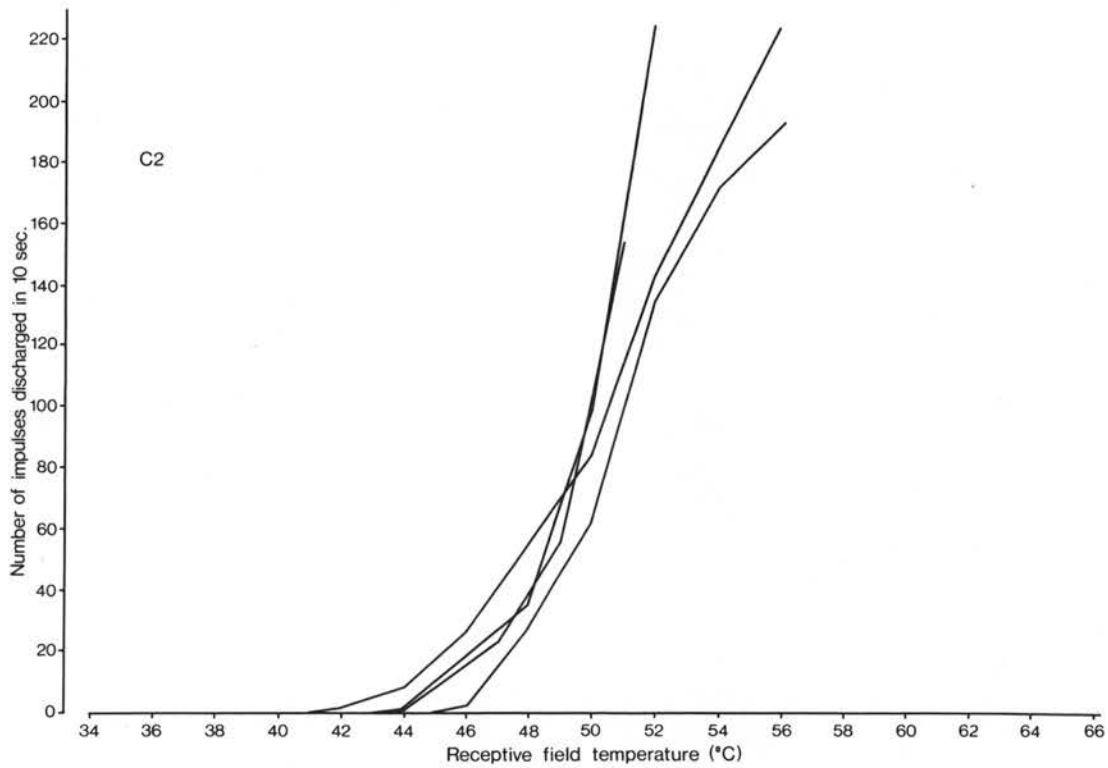

C2
Number of impulses discharged in 10 sec.
0
20
40
60
80
100
120
140
160
180
200
220
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
66
Receptive field temperature (°C)

Fig 2:4. Distribution of temperatures at which the heat-sensitive nociceptors displayed their peak or maximal response. Twenty five units showed a peak response within the range of temperatures tested (solid blocks). Eleven units did not show a peak response within the range of temperatures tested, and the highest temperatures tested on these units are included (open blocks).

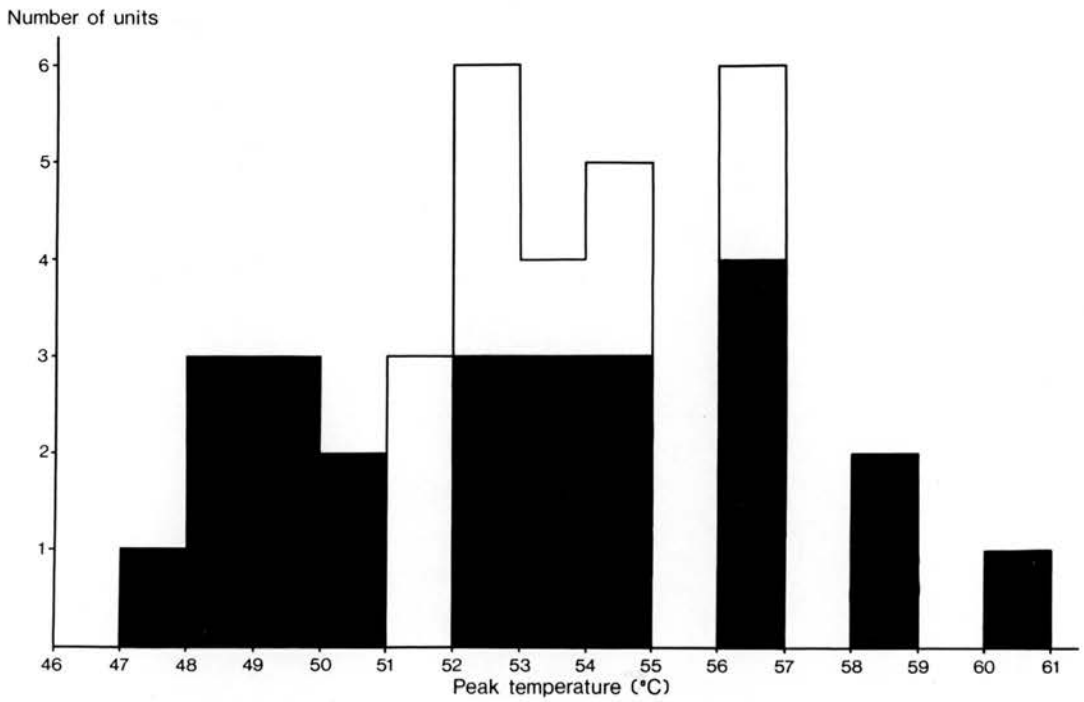

Number of units
6
5
4
3
2
1
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
Peak temperature (°C)

Fig 2:5 Peak/maximal responses of the 36 heat-sensitive nociceptors recorded from the beaks of normal birds. Twenty-five units showed a peak response within the temperature range tested (solid blocks). Eleven units did not show a peak response within the temperature range tested. The maximal responses recorded from these eleven units are included (open blocks)

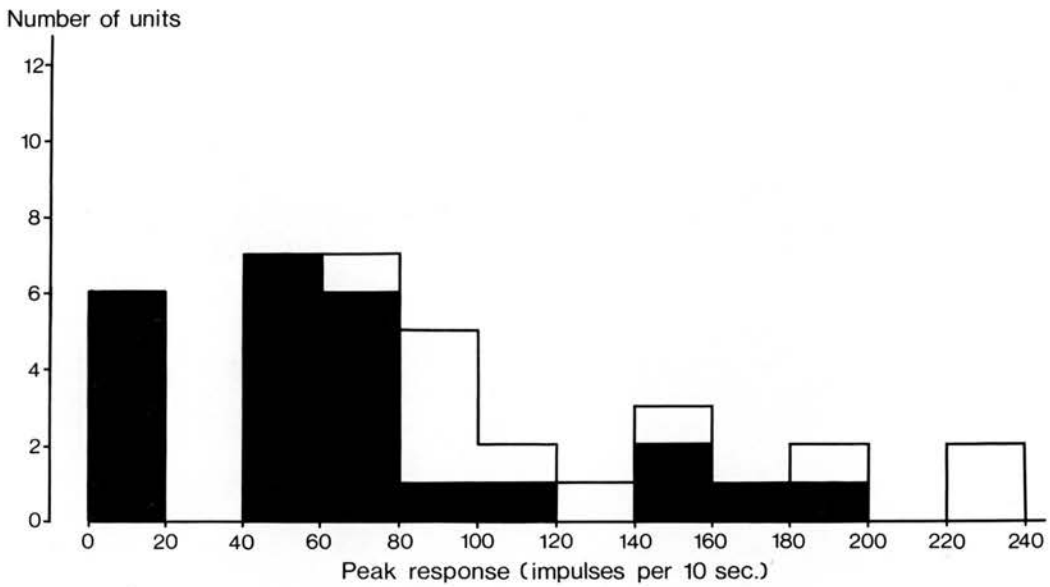

Number of units
12
10
8
6
4
2
0
0
20
40
60
80
100
120
140
160
180
200
220
240
Peak response (impulses per 10 sec.)

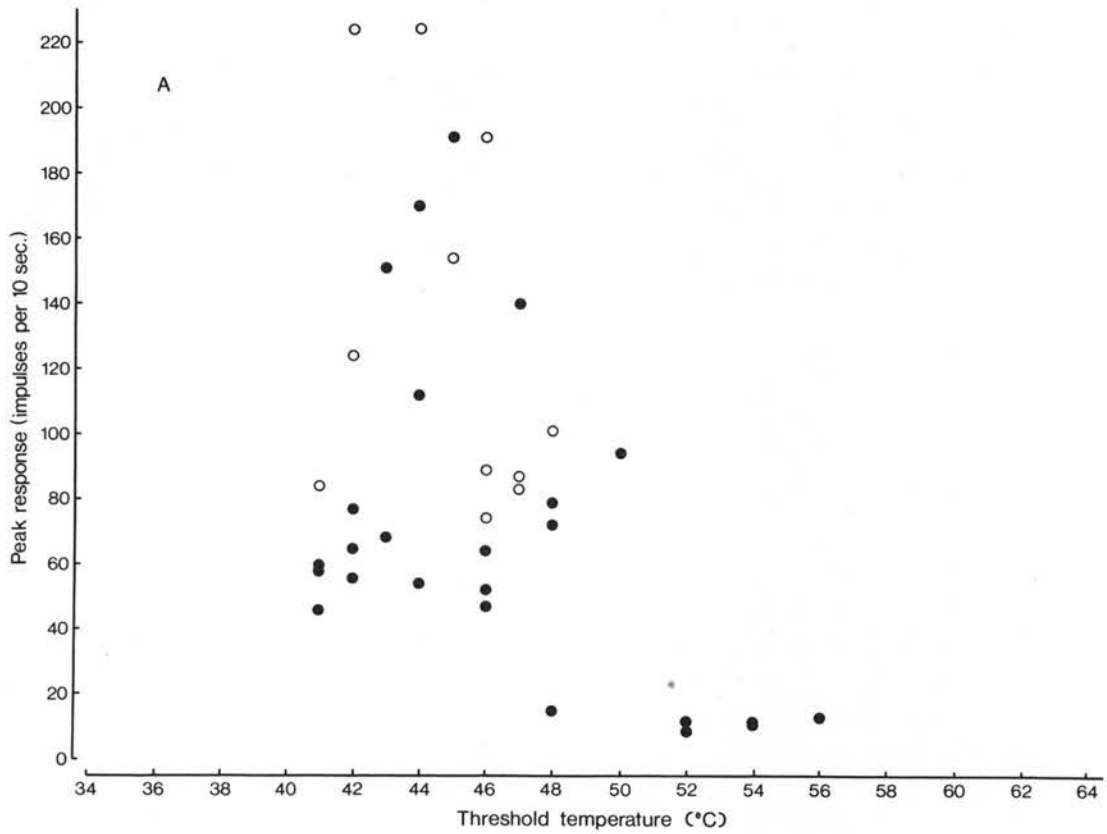
Fig 2:6
A. Illustrates the relationship between the peak/maximal response and the threshold temperature for the 36 heat-sensitive nociceptors.

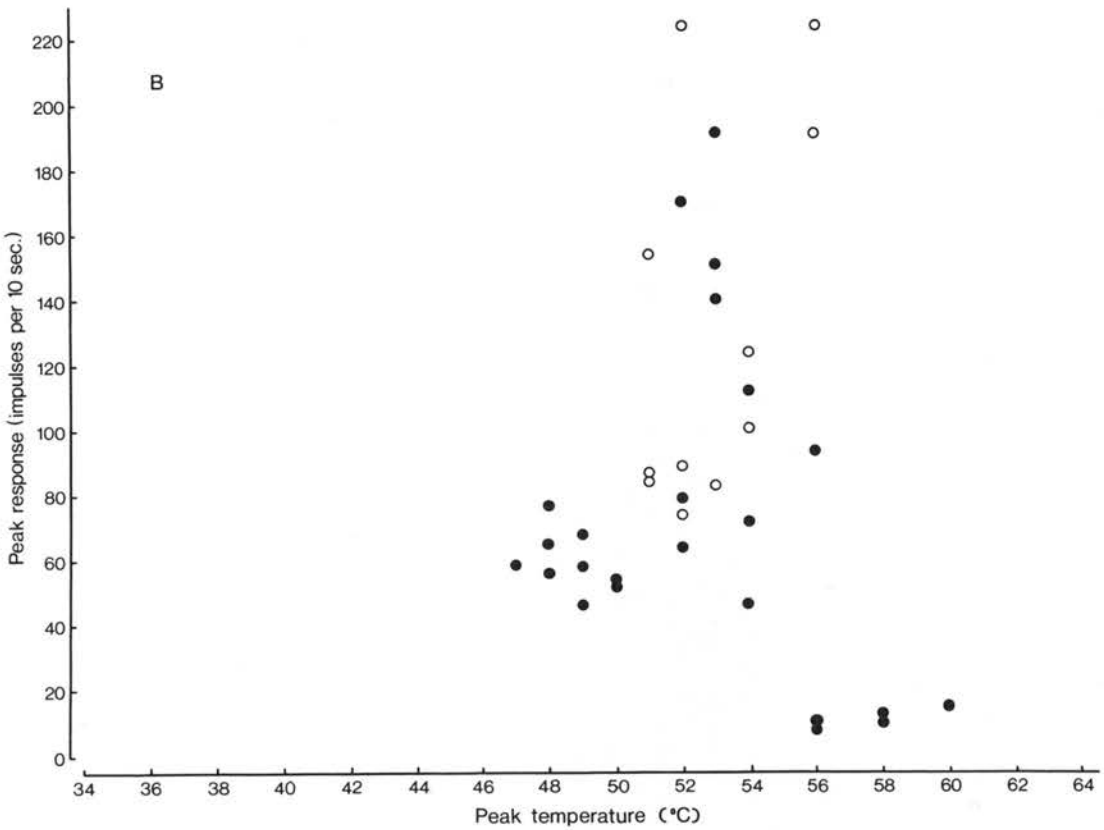
B. Illustrates the relationship between the peak/maximal response and the peak/maximal temperature for the 36 heat sensitive nociceptors.

● and ● represent one and two units respectively of the 25 units which showed a peak response.

o represents one unit of the 11 units for which a maximal response was obtained.

Note that no simple correlation can be detected in either case.


A
Peak response (impulses per 10 sec.)
0
20
40
60
80
100
120
140
160
180
200
220
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
Threshold temperature (°C)


B
Peak response (impulses per 10 sec.)
0
20
40
60
80
100
120
140
160
180
200
220
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
Peak temperature (°C)

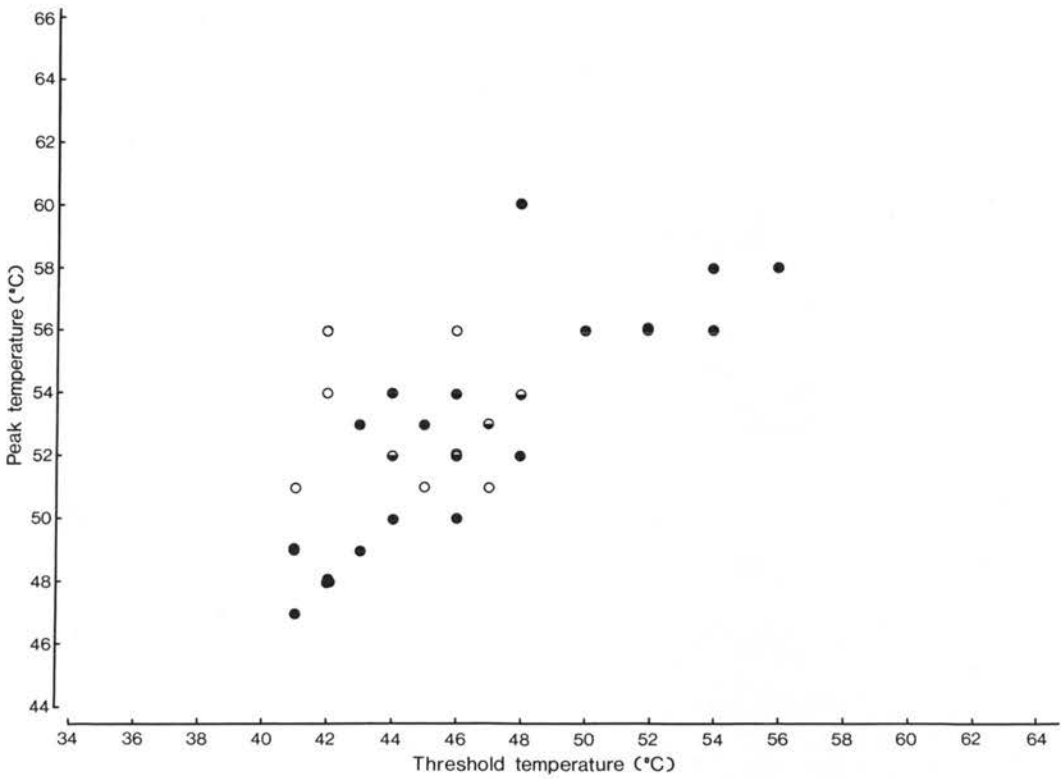
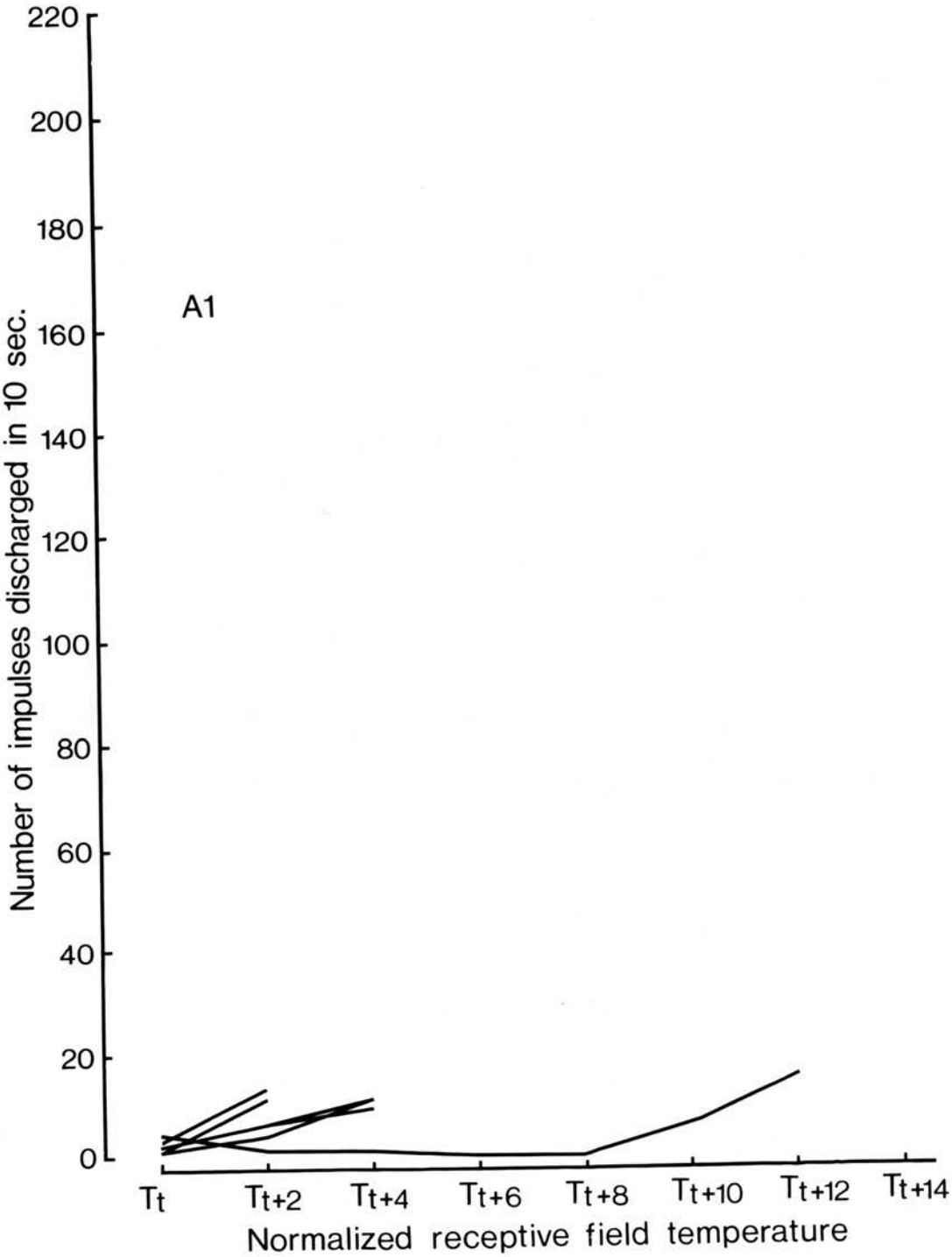
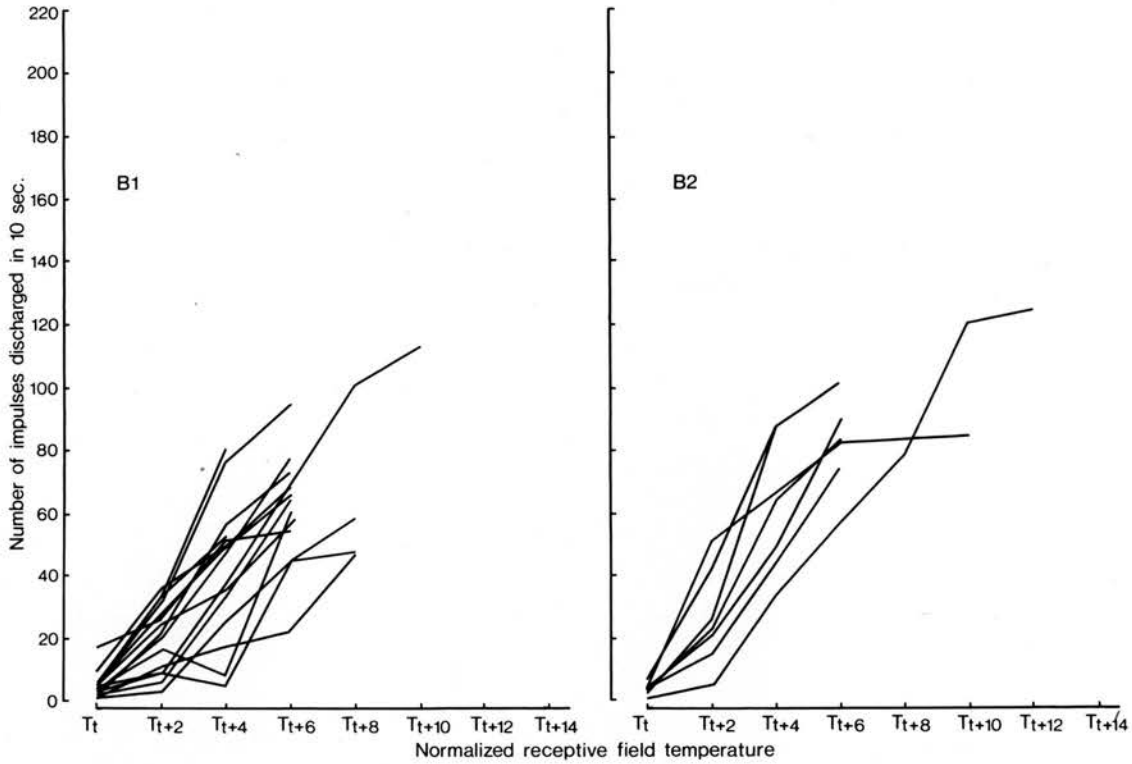

Peak temperature (°C)
66
64
62
60
58
56
54
52
50
48
46
44
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
Threshold temperature (°C)

Fig. 2:8 The stimulus-response curves for the 36 heat-sensitive nociceptors recorded from the beaks of normal birds. The portion of each curve from threshold to peak/maximal response is shown. The stimulus temperatures have been normalized, to facilitate a visual comparison of the thermal ranges and sensitivities of the units. Normalization was carried out with respect to threshold, with Tt representing threshold, Tt+2 2 °C above threshold, Tt+4 4°C above threshold, etc..

The stimulus-response curves have been divided into 5 groups in the same manner as shown in Fig. 2:3. Groups A1, B1 and C1 contain the units which had peak discharges of < 20 impulses per 10 sec., 40 to 130 impulses per 10 sec., and >140 impulses per 10 sec., respectively. Groups B2 and C2 contain the units which had maximal discharges of 20 to 130 impulses per 10 sec. and >140 impulses per 10 sec. respectively.


220
200
180
160
140
120
100
80
60
40
20
0
A1
Number of impulses discharged in 10 sec.
Tt
Tt+2
Tt+4
Tt+6
Tt+8
Tt+10
Tt+12
Tt+14
Normalized receptive field temperature


B1
B2
Number of impulses discharged in 10 sec.
220
200
180
160
140
120
100
80
60
40
20
0
Tt
Tt+2
Tt+4
Tt+6
Tt+8
Tt+10
Tt+12
Tt+14
Normalized receptive field temperature

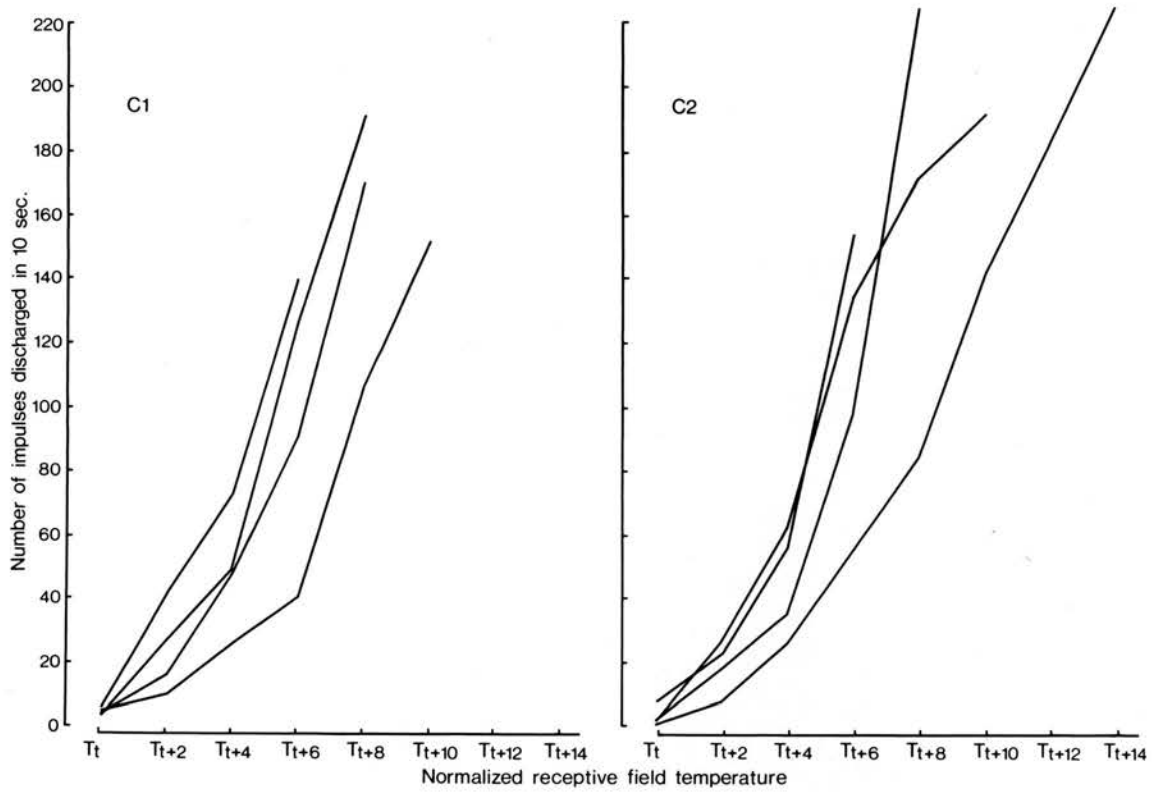

C1
C2
Number of impulses discharged in 10 sec.
220
200
180
160
140
120
100
80
60
40
20
0
Tt
Tt+2
Tt+4
Tt+6
Tt+8
Tt+10
Tt+12
Tt+14
Normalized receptive field temperature

Fig. 2:9 Reproducibility of the stimulus-response curves, illustrated by the responses of three heat sensitive nociceptors to repeated testing at each stimulus temperature.

● Indicates the responses to the first series of stimuli,
o the responses to the second series of stimuli and
▲ the responses to the third series of stimuli.

Within each stimulus series the suprathreshold stimuli were delivered in random order. The time interval between stimuli and between each series was 3 minutes.

Unit 26. The receptive field of this unit was located 5mm proximal to the tip of the beak. The mechanical threshold was 21g.

Units 34 and 36. The receptive fields of these units were located 3mm and 6mm proximal to the tip of the beak respectively. Neither unit was mechanically sensitive.

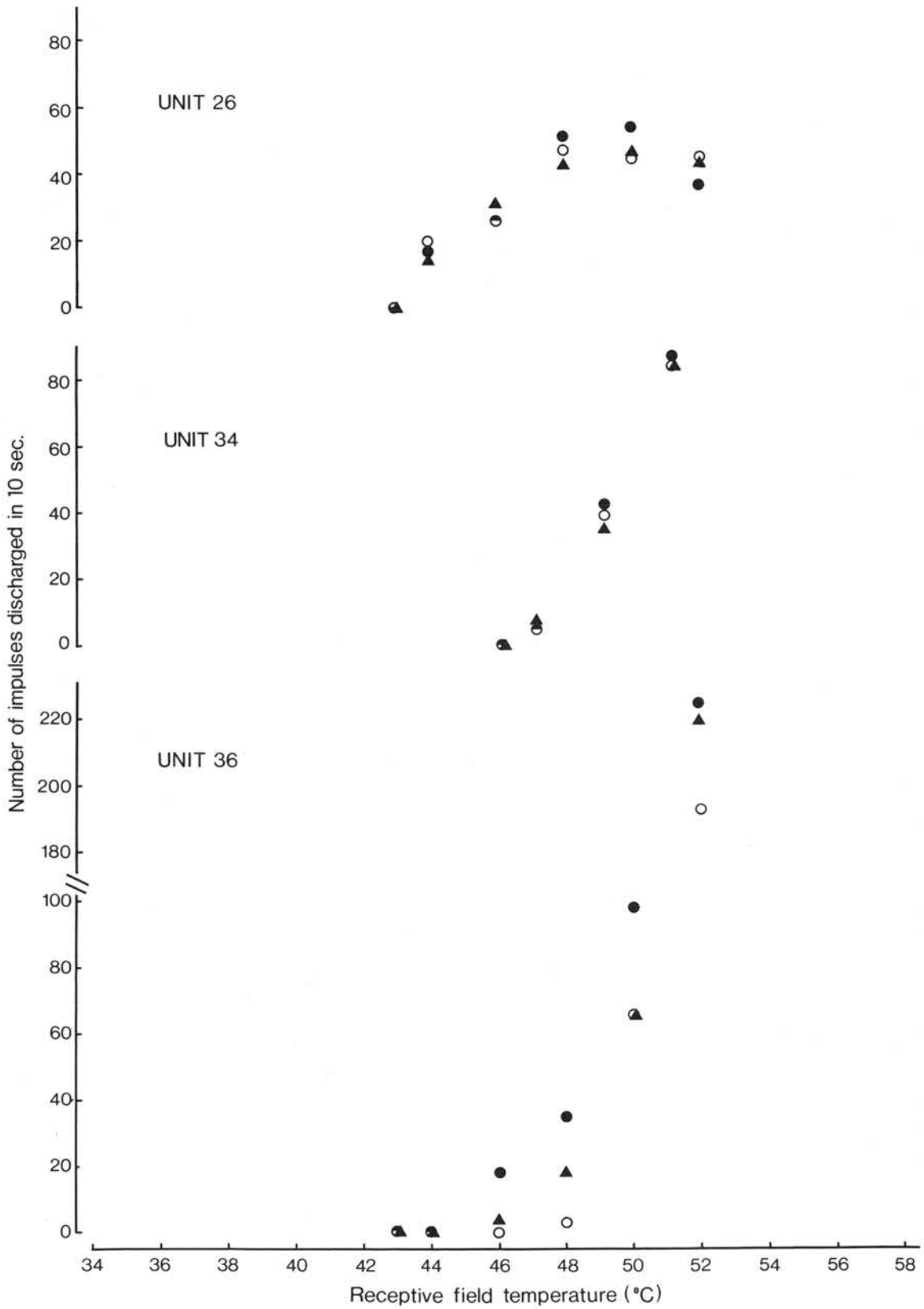

UNIT 26
UNIT 34
UNIT 36
Number of impulses discharged in 10 sec.
Receptive field temperature (°C)
0
20
40
60
80
100
180
200
220
34
36
38
40
42
44
46
48
50
52
54
56
58

Fig. 2:10. The stimulus-response relationship for unit 36. Each data point represents the arithmetic mean of the three separate response values illustrated in Fig. 2:9. The data are shown plotted out on linear axes (A) untransformed, (B) after log transformation of the stimulus and (C) after log transformation of both stimulus and response.

The figure provides a visual impression of goodness-of-fit of the data, i.e. the nearest approximation to a straight line relationship, to different intensity functions. The three intensity functions tested here are, after transformation to linear axes,

(A) Linear, $R=b.S + A$
(B) Logarithmic, $R=b.\log S + A$
(C) Power, $\log R=b.\log S + \log A$

where R= response magnitude
b= slope
S= stimulus intensity
A= intercept

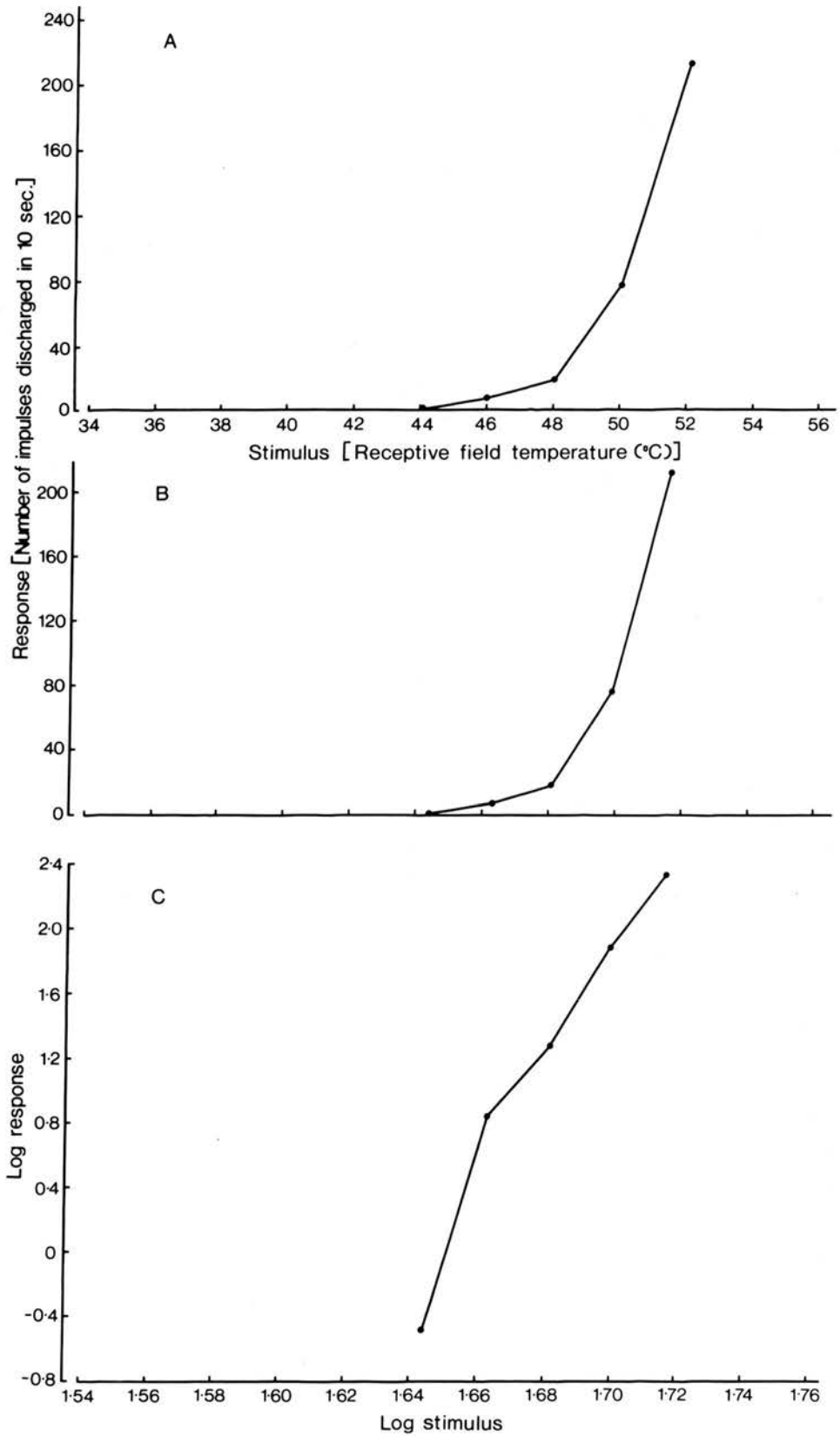

A
240
200
160
120
80
40
0
34
36
38
40
42
44
46
48
50
52
54
56
Stimulus [Receptive field temperature (°C)]
Response [Number of impulses discharged in 10 sec.]
B
200
160
120
80
40
0
C
2·4
2·0
1·6
1·2
0·8
0·4
0
-0·4
-0·8
Log response
1·54
1·56
1·58
1·60
1·62
1·64
1·66
1·68
1·70
1·72
1·74
1·76
Log stimulus

Fig. 2:11 The stimulus-response data of unit 36 plotted out in the same manner as in Fig. 2:10 after normalizing the stimulus temperature values. The normalization was carried out with respect to threshold, by assigning the threshold temperature (Tt) the numerical value of 1, thus Tt=1, Tt+2=3, Tt+4=5, etc.. The three intensity functions tested, after normalization and transformation to linear axes, are:

(A) Linear, $R=b.(S-S_o) + A$
(B) Logarithmic, $R=b.\log(S-S_o) + A$
(C) Power, $\log R=b.\log(S-S_o) + \log A$

where S_o = highest subthreshold stimulus intensity, i.e. 1°C below threshold.

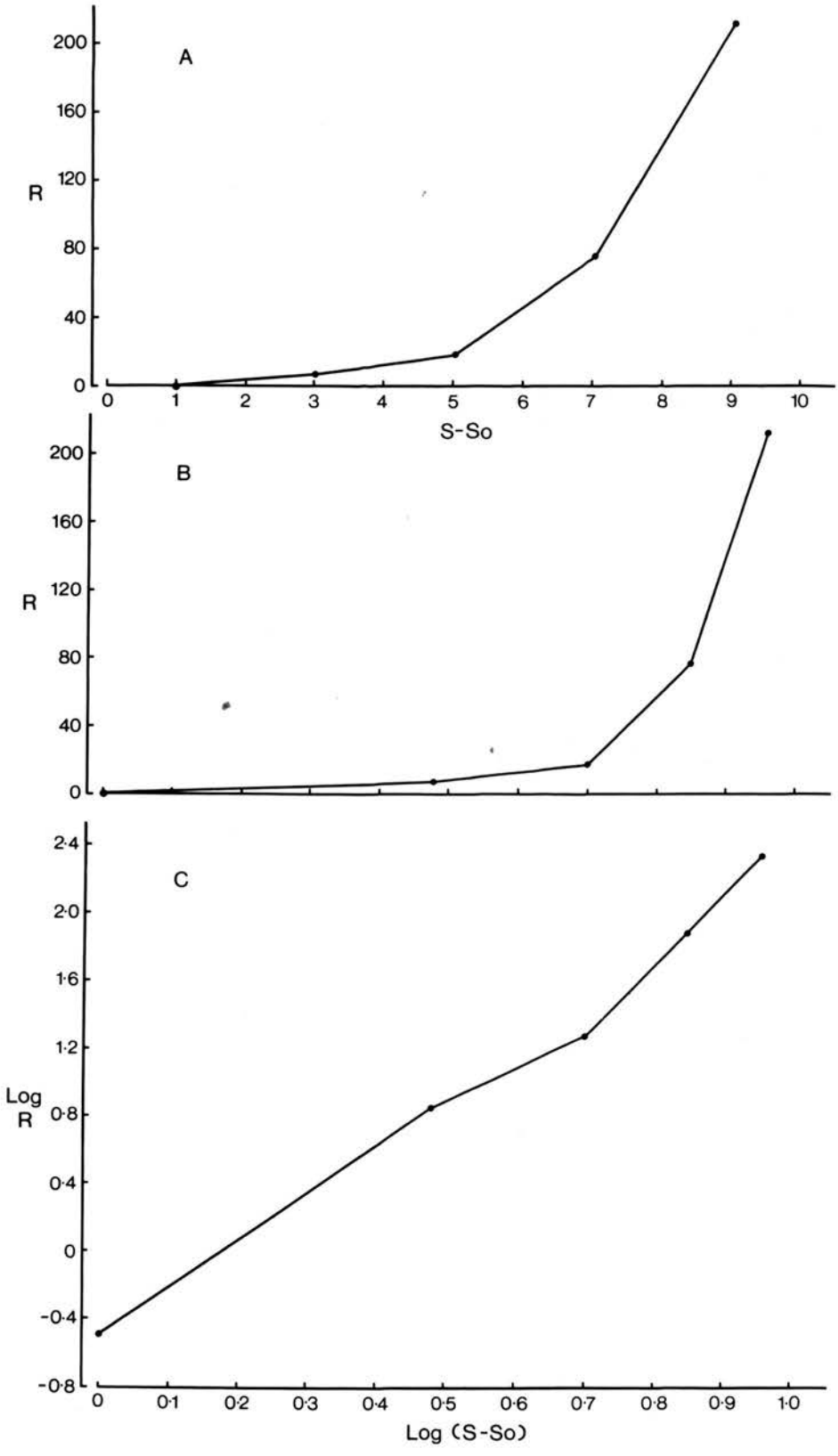

A
R
200
160
120
80
40
0
0 1 2 3 4 5 6 7 8 9 10
S-So
B
R
200
160
120
80
40
0
C
Log R
2·4
2·0
1·6
1·2
0·8
0·4
0
-0·4
-0·8
0 0·1 0·2 0·3 0·4 0·5 0·6 0·7 0·8 0·9 1·0
Log (S-So)

Fig 2:12 The stimulus-response data of unit 36 plotted out in the same manner as Fig. 2:11, with the calculated regression lines fitted through the data points. The correlation coefficients for the regression lines are, respectively,

(A) Linear, 0.8791
(B) Logarithmic, 0.7183
(C) Power, 0.9938

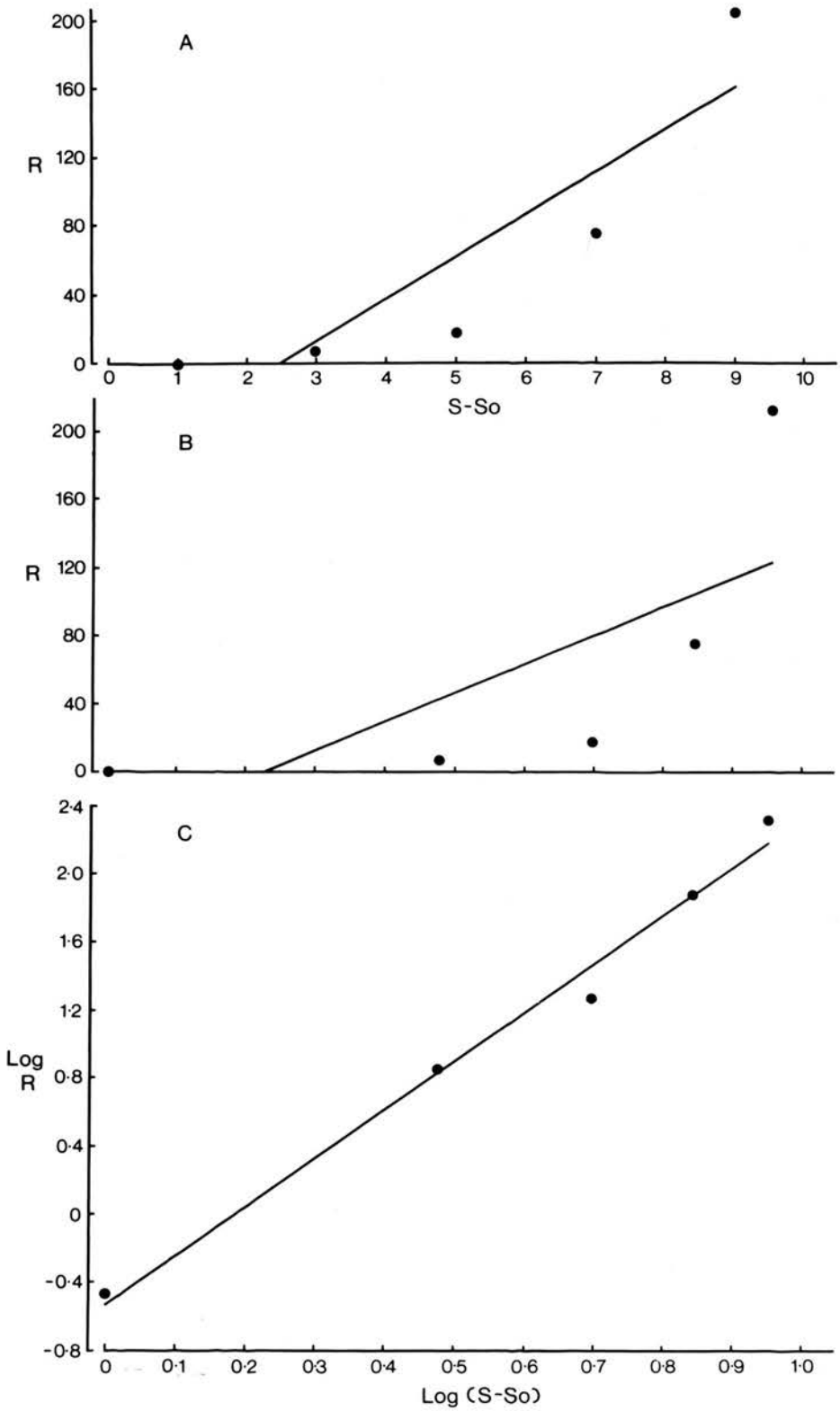

A
R
200
160
120
80
40
0
0 1 2 3 4 5 6 7 8 9 10
S-So
B
R
200
160
120
80
40
0
C
Log R
2·4
2·0
1·6
1·2
0·8
0·4
0
-0·4
-0·8
0 0·1 0·2 0·3 0·4 0·5 0·6 0·7 0·8 0·9 1·0
Log (S-So)

Fig. 2:13. The sensitivities of the heat-sensitive nociceptors recorded from the beaks of normal birds. The values for the sensitivities were derived from the regression lines fitted to the power-transformed stimulus-response data. Solid blocks represent the units which showed a peak response within the range of temperatures tested (n=25). Open blocks represent the units which did not show a peak response within the range of temperatures tested.

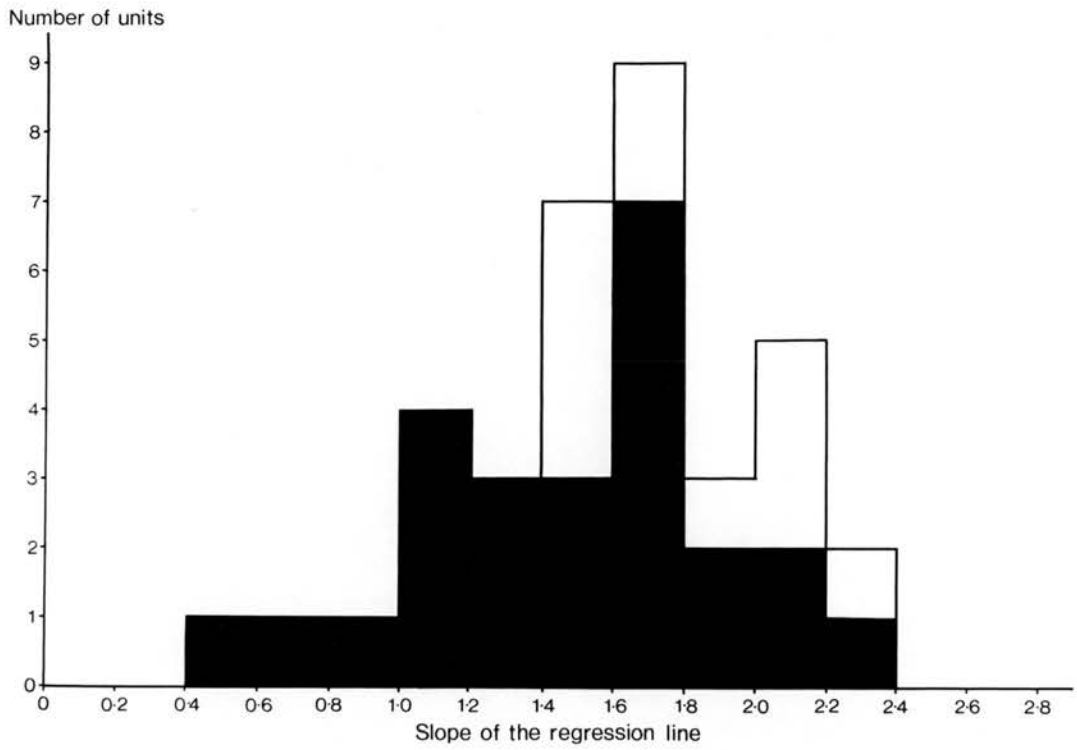

Number of units
9
8
7
6
5
4
3
2
1
0
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
1·8
2·0
2·2
2·4
2·6
2·8
Slope of the regression line

Table 2:1. The raw data from which the stimulus response curves illustrated infig. 2:3 were constructed. The values in the body of the table are the numbers of impulses discharged during the 10 second hold phase of a ramp and hold heat stimulus. The temperatures of the stimuli are displayed on the top of the table. Each row contains the stimulus-response data for one unit.

NUMBER OF IMPULSES DISCHARGED IN 10 SECONDS

Unit Number	Temperature (°C)																					
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
1	5		9		5		44		58		51													
2		4		24		35		56		39														
3		3		20		47		77		71														
4			3		28		49		68		60													
5						6		33		52		50												
6						4		21		49		89												
7						4		15		44		74												
8						1		3		25		44		47		46								
9							3		23		64		83											
10		1		5		34		57		78		120		124										
11			5		10		26		40		106		151		150									
12				4		16		47		90		170		159										
13				3		29		37		69		100		112		110								
14					8		23		56		154													
15					4		27		49		126		191		187									
16						2		27		62		134		171		191								
17								3		33		79		75										
18								2		26		87		101										
19								1		21		56		72		64								
20												2		6		9		3		1				
21														2		6		11		8		2		1
22														1		11		1		3		1		
23																3		13		5		3		2
24										3		32		76		94		89						
25		1		8		26		55		84		142		183		224								
26				17		26		51		54		37												
27	1		11		17		22		46		33													
28	4		16		8		59		57		57													
29							6		42		72		140		130									
30								4		1		1		0		0		6		15		5		11
31												1		4		11		7		7		5		4
32		9		36		49		65		60														
33	4		51				82				84													
34							7		42		87													
35						2		6		33		64		61		55		50						
36				1		18		35		98		224												

Table 2:2. Stimulus-response characteristics of 25 units which exhibited a peak response within the range of heat stimuli tested.

Unit	Threshold Temperature Tt(°C)	Peak Temperature Tp(°C)	Temperature Range Tp-Tt(°C)	Peak Response (Impulses per 10 seconds)
1	41	49	8	58
2	42	48	6	56
3	42	48	6	77
4	43	49	6	68
5	46	50	4	52
8	46	54	8	47
11	43	53	10	151
12	44	52	8	170
13	44	54	10	112
15	45	53	8	191
17	48	52	4	79
19	48	54	6	72
20	52	56	4	9
21	54	58	4	11
22	54	56	2	11
23	56	58	2	13
24	50	56	6	94
26	44	50	6	54
27	41	49	8	46
28	41	47	6	59
29	47	53	6	140
30	48	60	12	15
31	52	56	4	11
32	42	48	6	65
35	46	52	6	64

Table.2:3. Stimulus-response characteristics of 11 units which did not exhibit a peak response within the range of heat stimuli tested. These 11 units displayed an increasing response up to the highest temperature tested.

Unit	Threshold Temperature Tt(°C)	Highest Temperature Th(°C)	Temperature Range Th-Tt(°C)	Maximal Response (Impulses/10 sec)
6	46	52	6	89
7	46	52	6	74
9	47	53	6	83
10	42	54	12	124
14	45	51	6	154
16	46	56	10	191
18	48	54	6	101
25	42	56	14	224
33	41	51	10	84
34	47	51	4	87
36	44	52	8	224

Table 2:4. Summary of the stimulus-response characteristics from tables 2 and 3.

a) Units which exhibited a peak response within the range of heat stimuli tested.

b) Units which did not exhibit a peak response within the range of heat stimuli tested, but which displayed an increasing response up to the highest temperature tested.

a)

Units Tested To Peak Response	Threshold Temperature Tt(°C)	Peak Temperature Tp(°C)	Temperature Range Tp-Tt(°C)	Peak Response (Impulses/10sec)
n	25	25	25	25
Range	41-56	47-60	2-12	9-191
Median	46	53	6	59
Mean	46.36	52.60	6.24	69.00
Standard Error	0.89	0.72	0.48	10.10

b)

Units Not Tested To Peak Response	Threshold Temperature Tt(°C)	Highest Temperature Tested Th(°C)	Temperature Range Th-Tt(°C)	Maximal Response (Impulses/10sec)
n	11	11	11	11
Range	41-48	51-56	4-14	74-224
Median	46	52	6	101
Mean	44.91	52.91	8.00	130.46
Standard Error	0.71	0.56	0.94	17.54

a and b)

All units	Threshold Temperature Tt(°C)	Peak or highest Temperature tested	Temperature Range	Peak or maximal Response (Impulses/10sec)
n	36	36	36	36
Range	41-56	47-60	2-14	9-224
Median	46	53	6	72
Mean	45.92	52.69	6.78	87.78
Standard Error	0.66	0.53	0.45	9.92

Table 2:5. Statistical parameters from the regression analysis of the stimulus-response relationship for 3 nociceptor units (26,34,36).

The intensity functions listed are those described in the text, i.e.:

Linear, $R = b.(S-So)+A$

Logarithmic, $R = b.\log(S-So)+A$

Power, $R = A.(S-So)^{b}$

r = correlation coefficient
b = slope of the regression line
A = intercept
P = significance level for r
NS = no significant difference from zero correlation ($P>0.05$).

Unit	Intensity Function	r	b	A	P(<)
26	Linear	0.9581	5.67	12.34	0.02
	Logarithmic	0.9551	39.49	15.05	0.02
	Power	0.9790	0.57	1.22	0.01
34	Linear	0.9948	19.67	-15.67	0.01
	Logarithmic	0.9523	105.43	2.00	0.05
	Power	0.9999	1.62	0.81	0.001
36	linear	0.8791	24.63	-60.30	0.05
	Logarithmic	0.7183	168.57	-37.45	NS
	Power	0.9938	2.85	-0.53	0.001

Table 2:6. Statistical parameters of the regression analysis of the stimulus response relationship for the 25 units which displayed a peak response within the range of heat stimuli tested. Symbols used in the table are the same as those used in table 2:5.

Unit	Intensity function	r	b	A	P
1	Linear	0.8913	7.05	-11.05	0.02
	Logarithmic	0.7494	49.64	-5.34	NS
	Power	0.7549	1.03	0.54	NS
2	Linear	0.9935	8.35	-3.65	0.001
	Logarithmic	0.9613	56.49	1.21	0.01
	Power	0.9912	1.34	0.64	0.001
3	Linear	0.9929	12.45	-13.05	0.001
	Logarithmic	0.9253	81.13	-4.23	0.05
	Power	0.9997	1.68	0.49	0.001
4	Linear	0.9980	10.80	-6.20	0.001
	Logarithmic	0.9786	74.05	-0.42	0.01
	Power	0.9874	1.63	0.54	0.01
5	Linear	0.9950	11.50	-4.17	0.01
	Logarithmic	0.9942	64.34	5.11	0.01
	Power	0.9934	1.38	0.80	0.01
8	Linear	0.9649	6.65	-9.25	0.01
	Logarithmic	0.8970	51.78	-6.81	0.02
	Power	0.9628	1.93	-0.11	0.01
11	Linear	0.9352	14.74	-32.12	0.01
	Logarithmic	0.7852	120.69	-24.47	0.05
	Power	0.9540	1.43	0.53	0.001
12	Linear	0.9544	20.30	-36.10	0.01
	Logarithmic	0.8286	147.58	-22.43	0.05
	Power	0.9903	1.69	0.53	0.001
13	Linear	0.9861	12.14	-17.86	0.001
	Logarithmic	0.9004	108.11	-17.38	0.01
	Power	0.9839	1.63	0.39	0.001
15	linear	0.9659	23.65	-38.85	0.01
	Logarithmic	0.8519	174.68	-24.55	0.05
	Power	0.9954	1.74	0.59	0.001
17	Linear	0.9927	19.00	-18.67	0.01
	Logarithmic	0.9464	101.44	-1.43	NS
	Power	0.9985	2.06	0.49	0.01
19	Linear	0.9901	12.40	-12.10	0.01
	Logarithmic	0.9551	83.64	-4.76	0.02
	Power	0.9866	2.28	0.08	0.01
20	Linear	0.9966	1.75	0.42	0.01
	Logarithmic	0.9921	9.76	1.84	0.01
	Power	0.9986	0.95	0.31	0.01

21	Linear	0.9979	2.25	-0.42	0.01
	Logarithmic	0.9633	12.16	1.57	0.05
	Power	0.9991	1.05	0.30	0.001
22	Linear	1.000	5.00	-4.00	0.001
	Logarithmic	1.000	20.96	1.00	0.001
	Power	1.000	2.18	-5.96	0.001
23	Linear	1.000	5.00	-2.00	0.001
	Logarithmic	1.000	20.96	3.00	0.001
	Power	1.000	1.34	0.48	0.001
24	Linear	0.9888	15.85	-12.15	0.01
	Logarithmic	0.9649	108.15	-3.40	0.01
	Power	0.9897	1.84	0.53	0.01
26	Linear	0.9588	6.80	9.80	0.02
	Logarithmic	0.9318	46.21	13.65	0.05
	Power	0.9629	0.63	1.20	0.01
27	Linear	0.9505	5.05	-5.85	0.01
	Logarithmic	0.8628	38.39	-3.45	0.05
	Power	0.9829	1.64	0.77	0.001
28	Linear	0.8002	7.85	-9.65	NS
	Logarithmic	0.6816	46.75	-1.87	NS
	Power	0.7938	1.07	0.58	NS
29	Linear	0.9816	21.60	-21.40	0.01
	Logarithmic	0.9104	140.07	-5.78	0.05
	Power	0.9964	1.59	0.80	0.001
30	Linear	0.6002	0.75	-1.39	NS
	Logarithmic	0.3452	4.80	0.34	NS
	Power	0.4504	0.51	0.17	NS
31	Linear	0.9744	2.50	-2.17	0.05
	Logarithmic	0.9070	13.03	0.23	NS
	Power	0.9931	1.45	-0.02	0.01
32	Linear	0.9866	9.05	3.55	0.01
	Logarithmic	0.9915	63.59	7.62	0.001
	Power	0.9893	1.02	0.99	0.01
35	Linear	0.9586	10.65	-16.35	0.02
	Logarithmic	0.8483	65.90	-7.05	NS
	Power	0.9642	1.79	0.20	0.01

Table 2:7. Statistical parameters of the regression analysis of the stimulus-response relationship for the 11 units which did not display a peak response within the range of heat stimuli tested. Symbols used in the table are identical to those of table 2:5.

Unit	Intensity Function	r	b	A	P(<)
6	Linear	0.9839	14.15	-15.85	0.01
	Logarithmic	0.9023	90.74	-5.10	0.05
	Power	0.9994	1.59	0.59	0.001
7	Linear	0.9822	11.95	-13.55	0.01
	Logarithmic	0.8971	76.32	-4.32	0.05
	Power	0.9923	1.51	0.56	0.001
9	Linear	0.9885	14.05	-12.95	0.01
	Logarithmic	0.9453	93.95	-4.22	0.02
	Power	0.956	1.77	0.50	0.001
10	Linear	0.9853	11.48	-20.52	0.001
	Logarithmic	0.8923	115.65	-24.91	0.01
	Power	0.9868	2.01	-0.04	0.001
14	Linear	0.9264	23.55	-33.95	0.05
	Logarithmic	0.8043	142.96	-11.99	NS
	Power	0.9679	1.44	0.83	0.01
16	Linear	0.9864	20.70	-26.37	0.001
	Logarithmic	0.9197	188.18	-28.15	0.01
	Power	0.9912	1.95	0.39	0.001
18	Linear	0.9718	17.90	-17.60	0.01
	Logarithmic	0.9381	120.82	-7.05	0.02
	Power	0.9904	2.11	0.35	0.01
25	Linear	0.9773	16.74	-43.58	0.001
	Logarithmic	0.8409	179.88	-51.44	0.01
	Power	0.9992	2.05	-0.02	0.001
33	Linear	0.9365	13.55	1.05	0.02
	Logarithmic	0.9899	100.14	4.65	0.01
	Power	0.9613	1.64	0.71	0.01
34	Linear	0.9974	20.00	-14.67	0.01
	Logarithmic	0.9611	107.92	3.03	0.05
	Power	0.9995	1.58	20.85	0.01
36	Linear	0.9149	26.30	-56.30	0.02
	Logarithmic	0.7711	185.63	-35.26	NS
	Power	0.9947	2.38	0.02	0.001

Table 2:8. Population correlation coefficient and coefficient of determination for each of the three intensity functions which were fitted to the data. For each function the relevant values of r and r^2 were pooled and the arithmetic mean calculated. The figures in the body of the table represent the mean $\pm$SE. The values are given for both the 25 units which displayed a peak response and for the 11 units which did not display a peak response, and for all 36 units.

POPULATION CORRELATION COEFFICIENT, r

Intensity Function	Units Tested to Peak Response	Units Not Tested to Peak Response	All Units
n	25	11	36
Linear	0.9542±0.0171	0.9682±0.0085	0.9585±0.0121
Logarithmic	0.8869±0.0280	0.8966±0.0202	0.8898±0.0202
Power	0.9487±0.0240	0.9889±0.0039	0.9610±0.0169

POPULATION COEFFICIENT OF DETERMINATION, r^2

n	25	11	36
Linear	0.9175±0.0281	0.9382±0.0163	0.9238±0.0200
Logarithmic	0.8054±0.0405	0.8079±0.0355	0.8061±0.0298
Power	0.9140±0.0366	0.9782±0.0076	0.9336±0.0258

IV. DISCUSSION

The results presented in this chapter demonstrate the presence in the chicken beak of cutaneous receptors which respond to potentially tissue damaging heat stimuli. These receptors have the ability to encode in their discharge information relating to the intensity of the heat. They may, therefore, be termed nociceptors in common with similar receptors described in mammals.

From the viewpoint of stimulus-specificity, the chicken nociceptors reported here appear to be of two types, heat-responsive and heat and mechanically-responsive. Chemical irritants were not employed in the present study, so it remains unknown whether the chicken nociceptors would respond to this mode of stimulus and thus qualify for the term polymodal nociceptors.

The properties of the chicken nociceptors will now be discussed with reference to the properties of other avian and mammalian nociceptors and their relevance to nociception and pain sensation assessed.

A comparison of the chicken nociceptors with other avian nociceptors

The present study can be compared with the only other

quantitative study of avian nociceptors, that of Necker and Reiner (1980) relating to nociceptors in the pigeon feathered skin. Their sample of 10 nociceptors included both heat-and heat and mechanically-responsive types. The range of heat threshold values (46 - 49 °C) reported by Necker and Reiner (1980) was much narrower, and the mean heat threshold (47.1°C) was slightly higher, than that obtained for the chicken nociceptors. These differences could be due to any one, or a combination of, a number of factors, e.g. sample size, differences in methodology, species- and /or location - specific differences in the receptors themselves.

The 3 heat stimulus-response curves obtained by Necker and Reiner (1980), which showed a positively accelerating increase in response with increase in temperature between 42 and 52°C, bear resemblance to some of the heat stimulus response curves curves obtained in the present experiments. Neither of the 3 nociceptors described by Necker and Reiner (1980) displayed a peak response within the temperature range tested. The maximal response they reported was approximately 2 impulses/sec. (mean frequency), which corresponds to the lowest peak response values found for the chicken nociceptors.

Necker and Reiner (1980) did not measure the conduction velocities of the afferent fibres innervating the pigeon nociceptors. However, they suggested that, on the basis of the

amplitude and waveform of the recorded action potentials, some of their nociceptors were innervated by A-delta and some by C fibres. In the present study all nociceptor conduction velocities measured fell into the C fibre conduction range. These measurements must, however, be regarded as an approximation, as direct stimulation of the afferent nerve fibres was not possible. Electrical stimulation of the receptive field probably results in an underestimate of the true conduction velocity of the axon, as the time taken for the stimulation current to reach the receptor and generate an impulse is unknown (Georgopoulos, 1976). In common with the observations of Necker and Reiner (1980) the amplitudes of the action potentials recorded from the chicken nociceptors were lower than those of the more easily isolated mechanoreceptors. However, extracellularly recorded action potential amplitude is not necessarily a reliable guide to the conduction velocity of the afferent fibres (Iggo, 1958) so caution must be exercised in interpreting this phenomenon.

A comparison of the chicken nociceptors with mammalian nociceptors

The properties of the nociceptors described in this study bear many similarities to those of the heat and mechanoheat nociceptors described in mammals. The mean (45.9°C) and range (41 to 56°C) of the heat thresholds reported here are close to those described for nociceptors in diverse body locations in

several species, by investigators employing different stimulus methodologies. The upper limit of the heat thresholds reported would seem to depend upon the highest temperatures tested by the investigators. Many investigators have not tested above 55 °C, perhaps missing some less sensitive units. Ranges of heat thresholds reported for the rat are large, i.e. 36-59°C (Lynn and Carpenter, 1982) and 30-55 °C (Fleischer et al, 1983). Those reported for the rabbit had the highest upper limit, i.e. 40-60°C (King et al, 1976) and 41-63 °C (Lynn, 1979). In between these two extremes lie the threshold values for the cat and monkey, i.e. (cat) : 42-56°C (Bessou and Perl, 1969), 40-55 °C (Beck et al, 1974), (monkey): 38-49°C (Beitel and Dubner, 1976a), 40-47°C (Croze et al, 1976), 41-53°C (Georgopoulos, 1976), 37-47°C (Dubner and Hu, 1977), 42-55 °C (Kumazawa and Perl, 1977) and 39 ->51 °C (LaMotte et al, 1982). The heat threshold values reported for the human nociceptors, obtained using the microneurographic technique introduced by Hagbarth and Vallbo (Hagbarth and Vallbo, 1967, Vallbo and Hagbarth, 1968), appear to have a more circumscribed range, eg. 40-47°C (Torebjork and Hallin, 1976), 41-43°C (LaMotte et al, 1982; Torebjork et al, 1984).

It is interesting to note that the authors of the study utilising the same type of stimulator and stimulus parameters to this study (although they employed a faster rise time for their stimulus pulses) reported almost identical heat thresholds

(40-55°C) for the C-fibre innervated heat nociceptors in the cat foot pad (Beck et al, 1974).

The discharge pattern of the majority of the chicken nociceptors during a sustained suprathreshold heat stimulus consisted of an irregular continuous discharge, the frequency of which appeared to increase then decrease during the stimulus. A similar discharge pattern has been described for mammalian nociceptors, e.g. in the cat (Iggo, 1959), monkey (Beitel and Dubner, 1976a; Croze et al, 1976) and human (Torebjork et al, 1984).

Some of the nociceptors described in the present study displayed peak responses at stimulus temperatures between 47 and 60°C, stimuli at intensities beyond the peak temperature producing a decreased response. The temperature at which the peak response occurs in other nociceptors has not been reported extensively. This is presumably due to the more restricted range of heat stimuli used by most authors, hence they didn't test to peak response. Beitel and Dubner (1976a) found that 3 units, out of 10 tested to 55°C, peaked between 50-53°C. Georgopoulos (1977) reported that 4 of his units, out of 15 tested to 53°C, peaked between 49-53°C. Handwerker and Neher (1976) reported that 13 of their units, out of 47 tested to 53°C, peaked between 48-50 °C. In the human, Torebjork et al (1984) recently reported that 4, out of their sample of 14 nociceptors tested to 51°C, peaked

at 49-51 °C.

The range of peak/maximal responses obtained in the present study (9 to 224 impulses per 10 sec.) can be compared with the highest, although not necessarily peak, responses obtained by other investigators. For example, Beck et al (1974), 15-80 impulses per 10 sec. for their C-fibre innervated nociceptors, Beitel and Dubner (1976a), 5-50 impulses per 3 sec. (C-fibre innervated), Georgopoulos (1976), 4-20 impulses per 1 sec. (A-delta -fibre innervated), Torebjork et al (1984), 8-25 impulses per 5 sec. (C-fibre innervated).

Heat stimulus-response coding in nociceptors

The variety of shapes of the individual heat stimulus response curves obtained in the present study has been noted previously for heat stimulus-response curves obtained for mammalian nociceptors. The majority of the stimulus-response curves obtained for the chicken nociceptors were non-linear, implying that the sensitivity of the nociceptors, i.e the change in response for a given change in temperature, was non-linear. The stimulus-response curves were reproducible, at least for the 3 units tested 3 times at each stimulus temperature.

The stimulus-response relationship for any sensory receptor

indicates the extent to which that receptor can discriminate between different stimulus intensities and supply information relating to stimulus intensity to the CNS. Attempts have been made, in the past few decades, to compare animal electrophysiological data and human psychophysical data to determine how far human perceptual capacity can be explained by the primary afferent input from receptors. Two general psychophysical laws, those of Fechner (1860) and Stevens (1957,1970,1971), have been the subject of scrutiny by sensory neurophysiologists. These laws state, respectively, that sensation is related to the logarithm of the stimulus intensity (Fechner) or that sensation is related to a power of the stimulus intensity (Stevens). Although the Power law of Stevens has proved to be generally applicable to the sensory estimation of many different stimulus modalities in psychophysical experiments, animal electrophysiological experiments have indicated that neither of these two laws is generally true for all cutaneous receptor types. Power functions have been found to fit the stimulus-response relationship in many cutaneous receptor types, e.g. the static and dynamic component of the response of the SA type I mechanoreceptor (Werner and Mountcastle, 1965; Iggo and Muir, 1969), the dynamic components of the response of hair follicle afferents (Brown and Iggo, 1967), the static and dynamic components of the SA type I and II mechanoreceptors associated with sinus hair follicles (Gottschaldt et al, 1973), the static

component of the response of C-mechanoreceptors (Iggo and Kornhuber, 1977), and the static component of the response of the SA mechanoreceptors in the ankle joint capsules of normal and arthritic rats (Guilbaud, Iggo and Tegner, 1985). Evidence has accumulated, however, which indicates that the stimulus-response relationship for a particular cutaneous receptor type may be described equally validly by different intensity functions. For example, the static and dynamic components of the response of the SA II mechnoreceptor were fitted equally well by log and power functions by Chambers et al (1972). Kenton and Kruger (1973) found that the static component of the response of both SAI and SAII mechanoreceptors could be described equally well by linear and power functions. Power, log and linear functions were found to be equally well fitted to the dynamic response of rapidly adapting mechanoreceptors in glabrous skin by Iggo and Ogawa (1977).

The attempt, in this present study, to test the goodness of fit of the three intensity functions to the nociceptor heat stimulus response curves, was subject to some constraint. Ideally, for curve-fitting procedures, a large sample of data should be collected for each curve. This ideal was not achievable for the present study, due to the difficulties in dissecting out single nociceptor units, and recording from them for prolonged periods. This difficulty has presumably been encountered by other investigators who have obtained heat

stimulus-response curves for mammalian nociceptors, because very few authors have reported more than single measurements of the response at each stimulus intensity tested. This technical limitation means that statistical inference is somewhat limited. The correlation coefficient has been used as a measure of goodness of fit. This is common practice, e.g. Werner and Mountcastle (1965), Gottschaldt et al, (1973), Beck et al (1974), Georgopoulos (1977), Guilbaud et al (1985). It has, however, been criticised (Kenton and Kruger, 1973) on the grounds that the correlation coefficient does not account for the range of residual errors. However, it can be used to compare the present data to previous work.

In the present study, the three units tested three times at each stimulus intensity were fitted best by the power function, but for the rest of the data it is obvious that no one particular function describes every stimulus-response curve. The majority (64%) of the units were fitted best by the power function. The linear function accounted for 20% of the units, and the logarithmic function 6%. Beck et al (1974) found a good fit to a linear function ($r>0.9$) for the 6 C-fibre nociceptors tested extensively (20 trials each), and for 12 other C-fibre nociceptors tested once at each stimulus intensity. Beck et al (1974) did not, however, mention assessing the goodness-of-fit of other intensity functions to their data, so it is difficult to assess this discrepancy between their

results and the present ones. A limited number of other authors have attempted to fit intensity functions to nociceptor heat-stimulus-response-curves. Lynn (1979) and Fleischer et al (1983), using data from ramp stimulation of rabbit and cat C-fibre nociceptors, found that individual units were fitted by an exponential (log response) function (Lynn), or that the mean curve of pooled data from individual units was best fitted by a power function (Fleischer et al, 1983). There is an interesting agreement between the data obtained from the studies of primate trigeminal nociceptors and the present data. Beitel and Dubner (1976a) found that, of the stimulus-response curves obtained for 8 C-fibre innervated nociceptors, 5 were best fitted by a power function, 2 by a linear function and 1 by a log function. Georgopoulos (1977), instead of looking at the best fit out of the three functions for each individual curve, used the somewhat different approach of pooling the values of r (coefficient of determination) obtained for each function for each curve and calculating the mean value of r for each function for the population of units. Interestingly, the results of this analysis were that the best fit for the population of units was the power function, followed by the linear and log function, in that order. A similar analysis in the present study, yielded comparable results i.e.:

Values of r^2 for :	Linear	Power	Log	Function
Georgopoulos (1977)	0.928±0.060	0.962±0.024	0.854±0.102	
Present study	0.924±0.020	0.934±0.026	0.806±0.030	

(All ± S.E.)

Care must be exercised in interpreting these results, however, as (i) stimulus methodologies were different, (ii) Georgopoulos (1977) tested up to 53°C only (iii) pooling of data, i.e. finding the best fitting function for a population of units rather than treating each unit individually has been done before (Werner and Mountcastle, 1965) but has been subject to criticism (Kenton and Kruger, 1973). Their criticism is that pooling of data from individual receptors carries an implicit assumption that there is a CNS code dependent upon a pooled input. This assumption therefore implies that the best and worst discriminative capacity of individual fibres may be ignored by the CNS in favour of a pooled average, and there is no proof that this is in fact true.

Georgopoulos (1977) did not record the values of correlation coefficients for each individual curve, so a comparison of the present data with his along these lines is not possible. Comparing the values for the pooled r^2 for the present data and for those of Georgopoulos (1977), although in both cases the power function has the highest value of r^2, in both cases the values for the power function are not substantially different from those of the linear function. The pooled data may

therefore be equally well described by either function.

Bearing in mind these limitations, the power-transformed data from the present study can be used to obtain an estimate of the sensitivity of each nociceptor to compare with Georgopoulos' (1977) estimate. The present values (0.51 to 2.38, mean 1.58 ± 0.07, median 1.63) encompass those of Georgopoulos (1977) (0.83 to 2.16, mean 1.23 ± 0.28, median 1.10). Georgopoulos (1977) did not list the individual values of the sensitivities, but stated that "for most the sensitivity was a little over 1.0". For the present data, the individual sensitivity values were over 1.0 for all except 3 units.

The relationship between nociceptor discharge and pain sensation

Georgopoulos (1977) found an interesting correspondence between his data and the psychophysical stimulus-response intensity functions for heat pain obtained by Adair, Stevens and Marks (1968). The range and mean exponent (sensitivity) of power functions fitted to the data of Adair et al (1968) were similar to those found by Georgopoulos (1977) for the primate nociceptors. As he pointed out, the experimental variables in these two studies were somewhat different, so caution must be used in interpreting this similarity. This correspondence between the psychophysical and neurophysiological data has, however, been interpreted by Mountcastle (1980) to mean that human estimation of the intensity of noxious stimuli can be accounted for by the events occurring in the nociceptive afferents. For Mountcastle (1980), "pain is a specific sensation, with its own particular sets of specific peripheral afferent fibres".

The activation of cutaneous nociceptors is held to be necessary for the conscious experience of pain in humans (e.g. Zimmerman, 1979; Kruger and Rodin, 1983; Lamotte, 1984; Perl, 1984) and recently attempts have been made, using combined microneurographical and psychophysical techniques to relate the discharges in individual nociceptive afferents to the concurrent conscious experience related by the human subjects

during noxious cutaneous stimulation (e.g. Gybels et al, 1979; LaMotte et al, 1982; Adriaensen et al, 1983; Torebjork et al, 1984). However, this 'specificity' theory of pain has been subjected to criticism by Wall and his co-workers (e.g. Wall and McMahon, 1985). They do not deny the specificity, or specialization as they term it (Melzack and Wall, 1983), of peripheral cutaneous receptors, but challenge the concept that primary afferent nociceptive activity is transmitted directly to the higher CNS without alteration. Their objection is primarily on the grounds that this concept does not explain many clinical types of chronic pain. In brief, they propose that complex interactions of different afferent inputs at segmental and suprasegmental levels lead to the experience of pain. (Melzack and Wall, 1965; Wall, 1976, 1978,1979,1980,1984a,b; Wall and Woolf, 1980). Whilst it is true that specifically nociceptive cells have been found at different levels of the CNS, including the somatosensory cortex (Kenshalo and Isensee, 1983), interaction and modulation of different inputs has also been observed (e.g. Handwerker, Iggo and Zimmermann, 1975). Much work is being done to resolve this fascinating problem.

The present results demonstrate that the chicken beak contains nociceptors which can detect and transmit information relating to the intensity of potentially noxious temperatures. The properties of these nociceptors resemble those described in

mammals. It is interesting that the properties of heat-sensitive nociceptors are very similar across species and body location, suggesting that heat nociceptors are a generally occurring receptor. Noxious stimuli provoke defence reflex reactions in animals, nocifensive reactions (Casey and Morrow, 1983). To what extent this implies conscious perception of pain is unknown. The question of pain perception in animals is the subject of much contemporary interest and debate, both in the scientific community and in society as a whole. There is as yet no definite answer to this problem; only humans can report subjective conscious experience. In animals behavioural tests can be used to infer subjective states (reviewed by Chapman, Casey, Dubner, Foley, Gracely, and Reading, 1985) and can be correlated with physiological parameters indicative of stress or pain (Duncan and Molony, 1985).

Very little, however, is known about the physiology of the chicken CNS. Electrophysiological studies on the pigeon trigeminal system have demonstrated that information from mechanoreceptors in the beak is transmitted to the main sensory trigeminal nucleus (Zeigler and Witkowsky, 1968), the spinal trigeminal nucleus (Silver and Witkowsky, 1973) and the nucleus basalis (Witkovsky, Zeigler and Silver, 1973). The nucleus basalis projects to the archistriatum and the suggestion has been made that these two structures may be homologous to the mammalian thalamic relay nuclei and the somatosensory cortex

(Necker, 1983).

The only data available on central representation of nociceptive information in birds comes from Gentle (1979a). He recorded multi-unit activity, in response to temperatures above 45 °C applied to the buccal epithelium, in the ventrolateral cell group of the medullary solitary complex of the chicken. This is thought to be a first order relay nucleus transmitting mechanical, thermal and gustatory information via the chorda tympani and glossopharyngeal nerves (Gentle, 1979b). In separate behavioural experiments Gentle (1979a) found a significant decrease in water intake of chickens when the drinking water temperature was increased to 45 °C, in agreement with Gates and Kare (1961). Necker (1977) observed that heating the beak of the lightly-anaesthetized (urethane) pigeon to 45°C caused openings of the beak and movements of the tongue. A similar phenomenon has been observed in the chicken (Breward, unpublished observations). Necker (1977) considered these movements to be a defence mechanism, and they are perhaps analagous to the jaw-opening reflex produced by tooth pulp stimulation in cats. Clearly in the light of the results presented in this chapter, it would be useful to obtain both electrophysiological and behavioural data on central processing of nociceptive information in the trigeminal system of the chicken.

The acute electrophysiological effects of beak trimming

The approach of a heated blade, such as is used in the process of beak trimming, to the beak of a chicken would be expected to activate the nociceptors described in the present study. The process would also affect cold receptors, decreasing their spontaneous activity. Mechanical contact with the beak would presumably stimulate the mechanoreceptors. A considerable amount of information would be transmitted from the beak regarding the stimulus as a result of these responses of these receptor types. This would be expected to occur no matter at what point along the beak the blade approached, as receptive fields were located all over the beak. As the blade cuts into the beak and the underlying nerves, an injury discharge from all axons would be expected (Adrian, 1930; Wall, Waxman and Basbaum, 1974; Devor and Bernstein, 1982). The next question is: what happens to the peripheral afferent input after beak trimming? The following section of this thesis addresses this question.

Chapter 3.

CUTANEOUS NOCICEPTORS IN THE TRIMMED BEAK OF THE CHICKEN

3. CUTANEOUS NOCICEPTORS IN THE TRIMMED BEAK OF THE CHICKEN

I. INTRODUCTION

The aims of the experiments described in this chapter were two-fold. Firstly, to describe any abnormal nervous activity arising from the beak after beak trimming. Secondly, to quantify any changes in the heat-stimulus response properties of nociceptors in trimmed beaks. It has been suggested that decreased heat thresholds observed in regenerating nociceptors in cats might provide a neurophysiological basis for the hyperpathia observed in man during nerve regeneration (Dickhaus, Zimmermann and Zotterman, 1976a,b). It was therefore of interest to determine whether a comparable phenomenon occurred in the present preparation.

II. METHODS

Animals

The experiments were performed on Brown Leghorn hens. The birds were hatched and reared at the A.F.R.C. Poultry Research Centre, Roslin. They were individually housed in cages and had free access to food and water. Beak trimming was carried out on birds 4 to 5 months old (Weight 0.8 to 1.5 kg).

Beak trimming procedure

The birds were anaesthetized with sodium pentobarbitone (Sagatal, May and Baker Ltd.), 60 mg./ml. solution, injected into the brachial vein. The response of individual birds to barbiturate anaesthesia was variable, and the injection was continued until surgical anaesthesia (abolition of the comb-pinch reflex) was achieved. Effective doses for individual birds ranged from 24 to 30mg.

The beak length was measured, and one third of the upper and lower beak was removed using a commercial heated blade debeaker (Cope and Cope Ltd.). The debeaker consisted of a lower metal bar on which the beak was placed, and a movable electrically heated upper blade. The upper blade was manually pushed against the beak, cutting through the beak and cauterizing the stump at the same time. The temperature of the blade, measured with an infrared radiometer (AGA, Thermopoint 80), was 180°C.

The birds were returned to their cages immediately after beak trimming. Detailed behavioural observations were made on each bird prior to and after beak trimming. These observations will be reported in detail elsewhere (Slee, Duncan and Breward, unpublished observations).

Electrophysiological experiments

At intervals ranging from 1 to 84 days after beak trimming, the birds were used in acute electrophysiological experiments. Anaesthesia, the surgical preparation, recording, stimulating and data analysis techniques were identical to those described in Chapter 2.

III. RESULTS

A total of 196 single units was recorded from filaments dissected from the alveolar mandibular nerve from 35 beak-trimmed birds. The units were classified into three main groups: mechanoreceptors, nociceptors and spontaneously active units. The properties of the nociceptors are discussed below, preceded by a summary of the observations made on the mechanoreceptors. The spontaneously active units are considered separately in chapter 4.

The sample was biased because once the identifying characteristics of each afferent unit type had been established, a deliberate search was made for nociceptors and spontaneously active units. The numbers of the different afferent unit types therefore do not represent their true proportions in the trimmed beak.

MECHANORECEPTORS

53 units were recorded which responded only to mechanical stimulation of the beak surface. No gross differences were observed between the properties of the mechanoreceptors recorded from these beak-trimmed birds and those recorded from the normal birds. The units could be divided into two groups, rapidly and slowly adapting mechanoreceptors, using the same criterion adopted for the mechanoreceptors recorded from the intact beak.

Rapidly adapting mechanoreceptors

Two groups of rapidly adapting mechanoreceptors were distinguished by the same criterion adopted for the mechanoreceptors recorded from the intact beak. Type (i) (19 units) responded with a train of impulses during the movement phase of a mechanical identation, whereas type (ii) (17 units) responded to a similar stimulus with only a few impulses during the onset and sometimes the removal of such a stimulus.

Mechanical force thresholds ranged from 0.3 to 50g for type (i) and 4 to 50g for type (ii).

Neither type (i) nor type (ii) responded to cold (down to 0°C) or heat (up to 60°C) stimulation of the receptive field.

Receptive fields were circular or elliptical in shape, with the longest diameter of each receptive field ranging from 1 to 10 mm for type (i) and 1 to 2 mm for type (ii). The receptive fields for both types were distributed even ly over the beak surface, down to the distal tip of the stump.

Type (i) units were recorded 6 to 64 days after beak trimming, and type (ii) units 17 to 64 days after beak trimming.

Slowly adapting mechanoreceptors

17 units were classified as slowly adapting mechanoreceptors. Like the slowly adapting mechanoreceptors recorded from the intact beak, none of the units carried a resting discharge. Sustained suprathreshold mechanical indentation of the beak surface produced a discharge which lasted for the duration of the stimulus. Mechanical force thresholds ranged from 0.3 to 107g. There was no response to cold (down to 0°C) or to heat (up to 60°C) stimulation of the receptive field.

Receptive fields were circular or elliptical in shape, the longest diameter of each receptive field ranging from 1 to 4 mm. The receptive fields were distributed evenly over the beak surface, down to the distal tip of the stump.

Slowly adapting mechanoreceptors were recorded 1 to 77 days after beak trimming.

NOCICEPTORS

Forty-seven units were classified as nociceptors. They were recorded at 6- 84 days after beak trimming. They had the following general characteristics. All forty-seven units responded to heating the receptive field. Thirty-seven of the units also responded to mechanical stimulation of the receptive field. No resting discharge was observed. None of the units responded to cooling the receptive field (down to 0°C). An investigation of the response of these nociceptors to heat stimulation was carried out, in a manner similar to that described in Chapter 2.

1. The response of the nociceptors to heat stimulation

(i) Heat thresholds

Heat thresholds were measured for 42 units. During the preliminary characterisation of the remaining 5 units, recording problems were encountered, due to fluid seepage from the nerve onto the recording platform. This fluid accumulated rapidly despite frequent aspiration, and repeatedly shorted out the recording electrode. Previous experience had indicated that any recording under these conditions was likely to be short-lived. Given the probability of a short recording time, the decision was made to obtain as much information as possible about the heat stimulus-response curve without attempting to accurately measure the heat threshold. The thresholds ranged from 36 to 58°C with a median value of 46°C and a mean of 46.64 +0.83°C(S.E.).

(ii) Stimulus-response curves -general features

Examples of the stimulus-response relationship are illustrated in figs 3:2A and 3:2C. The discharge pattern of the unit illustrated in fig 3:2A is typical of the majority of the units recorded, i.e. an irregular continuous discharge, an increase in stimulus intensity producing an increased number of impulses discharged. Of the 47 units, 29 (61.7%) displayed this type of

discharge pattern. Eighteen units (38.3%) displayed the type of discharge exemplified by the unit illustrated in fig3:2c, i.e. a distinctive bursting discharge, the impulses occurring in clusters rather than as a continuous train.

Figs.3:2B and 3:2D illustrate the responses of the two units, plotted against receptive field temperature.

The stimulus-response data for all 47 units is presented in table 3:1 and is illustrated graphically in Fig. 3:3. Like the nociceptor units recorded from the intact beak described in chapter 2, these 47 units displayed an increased response with increased temperature. There was variability in the response characteristics i.e. threshold temperature, response magnitude, shape and slope of the curves and the range of temperatures over which the units responded. A peak response was elicited for 29 units (61.7%). Increasing the temperature beyond this peak produced a decrease in the response. The remaining 18 units (38.3% of the sample) showed an increasing response up to the highest temperature tested. Adopting the convention used in chapter 2, the response at the highest temperature tested for these 18 units is referred to as their maximal response.

The heat threshold, the temperature for the peak or maximal response, the temperature range from threshold to peak/maximal response and the amplitude of the peak/maximal response for all

47 units are presented in tables 3:2 and 3:3 and summarised in table 3:4.

(ii) Peak/Maximal temperature

The distribution of the temperatures at which the 47 units showed a peak/maximal response is illustrated in fig 3:4. Of the 29 units which displayed a peak response, 23 peaked at temperatures between 44 and 56°C. The remaining 6 of these 29 units peaked between 58 and 63°C.

Of the 18 units which displayed an increase in response up to the highest temperature tested, 13 where tested up to 48 to 56 °C and five were tested up to 58 to 65°C.

(iv) Peak/Maximal responses

The distribution of the peak/maximal responses for the 47 units is illustrated in fig.3:5. The 29 peak responses ranged from 4 to 196 impulses per 10 sec. The median value was 45 impulses per 10 sec, mean 53.31 $\pm$8.30 (S.E.). The majority (24) of the units had peak responses ranging from 4 to 70 impulses per 10 sec. The peak responses of the remaining 5 units ranged from 89 to 196 impulses per 10 sec.

The maximal responses of the 18 units with no

peak response ranged from 13 to 160 impulses per 10 sec.. The median value was 67 impulses per 10 sec., mean 68.83 ±9.51(S.E.). The majority (14) of the units had maximal responses ranging from 13 to 74 impulses per 10 sec.. The remaining 4 units had maximal responses ranging from 109 to 160 impulses per 10 sec..

The relationship between the peak/maximal response and threshold temperature, and between peak/maximal response and peak/maximal temperature is illustrated in fig. 3:6. Note that no simple correlation was detected in either case.

(v) Thermal range

The relationship between threshold temperature and peak/maximal temperature is illustrated in fig. 3:7. Again, as shown by the intact beak nociceptors, a positive trend seems to hold for this relationship. In general, the units with the lowest peak/maximal temperatures had the lowest threshold temperatures and units with the highest peak/maximal temperatures had the highest threshold temperatures. Using the definition of thermal range established in Chapter 2, the thermal ranges for the 25 units for which threshold and peak measurements were made varied from 2 to 12°C, median of 6 °C and mean of 6.40± 0.62 °C(S.E.). The thermal ranges for the 17 units for which threshold and maximal measurements were obtained varied from

6-14°C, median 12°C and mean of 10.94±0.57°C(S.E.).

The thermal ranges of all 42 units are illustrated graphically in fig. 3:8 after normalizing to heat threshold.

(vi) Repeatability of the stimulus-response curves

The heat stimulus-response curves obtained for the 47 units from the trimmed beak were, in general, non-linear (fig.3:3). Like the stimulus-response curves obtained for the intact-beak units, some of the curves are positively accelerating, some are negatively accelerating and some are sigmoid in shape. The repeatability of the shape of the stimulus-response curves was tested for four of the units in the manner described in chapter 2. The results are illustrated in fig. 3:9. For three of the units (58,60 and 61) no pattern could be detected in the change in responsiveness from trial to trial, although two of the units (58 and 60) showed a reduced response to the highest temperature tested in the third stimulus trial. The fourth unit (70) showed no change in threshold but did show a progressive attenuation of the response to suprathreshold temperatures from the first to the third trial.

(vii) Stimulus-response coding

The process of testing the goodness of fit of the three

intensity functions to the heat stimulus-response curves was carried out as described for the intact beak nociceptors in chapter 2. The data from unit 58 is used here as an illustrative example.

A visual impression of goodness of fit for each of the three intensity functions tested can be gained from fig. 3:10. The untransformed stimulus-response curve is curvilinear and positively accelerating. Logarithmic transformation of the stimulus increases the curvature, and logarithmic transformation of both stimulus and response substantially straightens out the curve.

The effect of normalizing the stimulus values and subsequently subjecting the data to logarithmic and power transformations is illustrated in fig 3:11. The logarithmic transformation extends the lower end of the curve and compresses the upper end, transformation of the stimulus alone producing a more pronounced curvature, whilst transformation of both stimulus and response results in a close approximation to a straight line. Figure 3:12 shows the calculated regression line fitted to the data of fig. 3:11. The correlation coefficients are 0.9338 for the untransformed data, 0.7778 for the stimulus-transformed data and 0.9779 for the stimulus- and response-transformed data. These correlation coefficients are significant at the 0.01, 0.05 and 0.001 levels, respectively.

Regression analysis was carried out on the data from units 60,61 and 70 after normalization and transformation as described above. The correlation coefficients are given in table 3:5. For units 60 and 61, the correlation was highest for the power function, the next best fit being the linear function in both cases. For unit 70 the correlation was highest for the linear function, the next best fit being the power function.

The stimulus-response data for the 42 single-trial curves (including the first stimulus trial for units 58,60,61 and 70) were subjected to the analysis described above. The summary statistics of these calculations are presented in tables 3:6 and 3:7. Out of the 25 units tested to peak response, and for which threshold measurements were obtained, 5 units (20%) showed no difference between the 3 functions. These were units 51,59,64,66, and 78, each with only two data points from threshold to peak response hence the correlation coefficient in each case is 1.0000. Two units (8%), 42 and 75, showed a poor correlation ($P>0.05$) with all three functions. Of the remaining 18 units, 8 (32%) showed the best fit to the linear function, 1 (4%) to the logarithmic function and 9 (36%) to the power function.

Of the 17 units not tested to peak response and for which threshold measurements were obtained, two units (11.8%), 40 and

55, showed a poor correlation (P>0.05) with all three functions. Of the remaining 15 units, 4 (23.5%) showed the best fit to the linear function, 2 (11.8%) to the logarithmic function and 9 (52.9%) to the power function.

Pooling the data for all 42 units, the number of units which show the best fit to a particular function are : 12 (28.6%) for the linear function, 3 (7.1%) for the logarithmic function, and 18 (42.9%) for the power function. 5 units (11.9%) showed no difference between the three functions, and 4 units (9.5%) showed a poor correlation with all 3 functions.

The best fitting function for the data as a whole, i.e. the population intensity function, was calculated as described in Chapter 2, and the results are given in table 3:8. For both the sample of units tested to peak response (n=25), the sample of units not tested to peak response (n=17), and for the whole population of units (n=42), the linear function was the best fit. Although, like the nociceptors described in Chapter 2, the difference between the goodness of fit of the linear and power functions was not great, and the population of units is probably equally well described by either.

(viii) Sensitivity

For the purpose of comparison with the data of Chapter 2, the sensitivities of the units were calculated from the power-transformed data. The values are given in tables 2:6 and 2:7 and are illustrated in fig.3:13. They ranged from 0.36 to 2.47 with a median of 1.25 and a mean of 1.27 ± 0.12 (S.E.) for the 25 units tested to peak response. For the data as a whole, i.e. adding on the 17 units not tested to peak response, the values ranged from 0.18 to 2.47 with a median of 1.30 and a mean of 1.26 ± 0.09 (s.e.).

2. Response to mechanical stimulation

Thirty seven of the 47 nociceptors also responded to mechanical stimulation of the receptive field. Mechanical force thresholds ranged from 1.4 to 50g, median 21g. Like the normal nociceptors, a sustained discharge was evoked during a suprathreshold mechanical stimulation of the receptive field.

3. Receptive fields

Receptive fields were circular or elliptical in shape, with the longest diameters of each receptive field ranging from 1 to 5 mm. They were distributed evenly over the beak surface, down to the distal tip of the stump.

4. Conduction velocities

Conduction velocities of the afferent fibres innervating the nociceptors ranged from 0.68 to 1.73 m/sec. (mean 1.17±0.12, median 1.30, n=10).

Comparison of the heat stimulus-response characteristics of nociceptors from normal and beak-trimmed birds

A comparison between the heat stimulus-response characteristics of the nociceptors in the intact beak and those in the trimmed beak was made. Parameters of the stimulus-response relationship examined for differences were heat threshold, peak/maximal temperature, peak/maximal response, thermal range and sensitivity. The significance of the differences observed was assessed using the Mann-Whitney U test.

There was no significant difference between the heat thresholds of the nociceptors from the intact beak and those from the trimmed beak ($P>0.2$).

Comparing the nociceptors tested to peak response from both the intact and trimmed beak, no significant difference was found for the peak temperature, peak response, thermal range and sensitivity ($P>0.1$, $P>0.07$, $P>0.3$, $P>0.1$ respectively). If the assumption is made that, within each of the two nociceptor

populations, the maximal response of the units not tested to peak approximate to their true peak values these units can be pooled with the units tested to peak response. A comparison can then be made between the pooled data for the intact beak nociceptors and that of the trimmed beak nociceptors, and significant differences appear. The peak/maximal temperatures in the trimmed beak are significantly higher, and their thermal ranges are larger than those in the intact beak ($P<0.03$ in both cases). The peak/maximal responses and sensitivities of the nociceptors in the trimmed beak are significantly lower than those of the intact beak ($P<0.006$ and $P<0.007$ respectively).

Fig. 3:1 Distribution of the heat thresholds of 42 heat sensitive nociceptors recorded from beak-trimmed birds. Each threshold was measured to the nearest 1°C. Range = 36 to 58°C, median 46°C, mean 46.64 ± 0.83°C(S.E.).

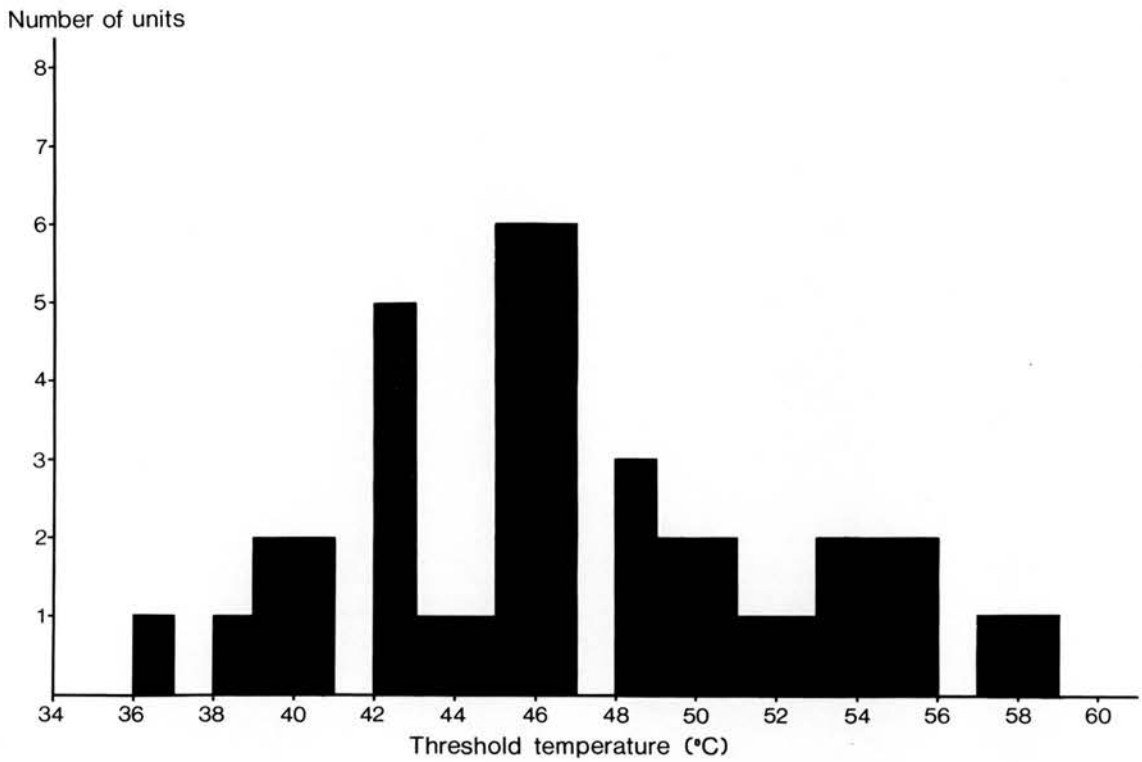

Number of units
8
7
6
5
4
3
2
1
34
36
38
40
42
44
46
48
50
52
54
56
58
60
Threshold temperature (°C)

Fig. 3:2 A. The afferent discharges of a cutaneous nociceptor recorded from the alveolar mandibular nerve of a beak-trimmed chicken, at different beak temperatures.

Specimen records. In each record, the lower trace is the action potential discharge and the upper trace is the beak surface temperature measured with a thermocouple positioned on the centre of the receptive field. The temperature of the receptive field was first raised to 34 °C, then ramp and hold heat stimuli at different temperatures were delivered. The stimuli were applied in random sequence, with three minutes between successive stimuli. The hold temperature of each stimulus was maintained for 10 sec.. The value of the hold temperature is indicated on the left of each record.

Note the increased discharge with increasing temperature. Unit no. 56. The receptive field of this unit was located 5mm. proximal to the tip of the trimmed beak. The receptive field was elliptical, 2 x 4mm., orientated with its long axis parallel to the long axis of the beak. Its mechanical threshold, measured with von Frey hairs, was 12g. This unit was recorded 18 days after beak amputation.

Calibration bar (time) = 10 sec.

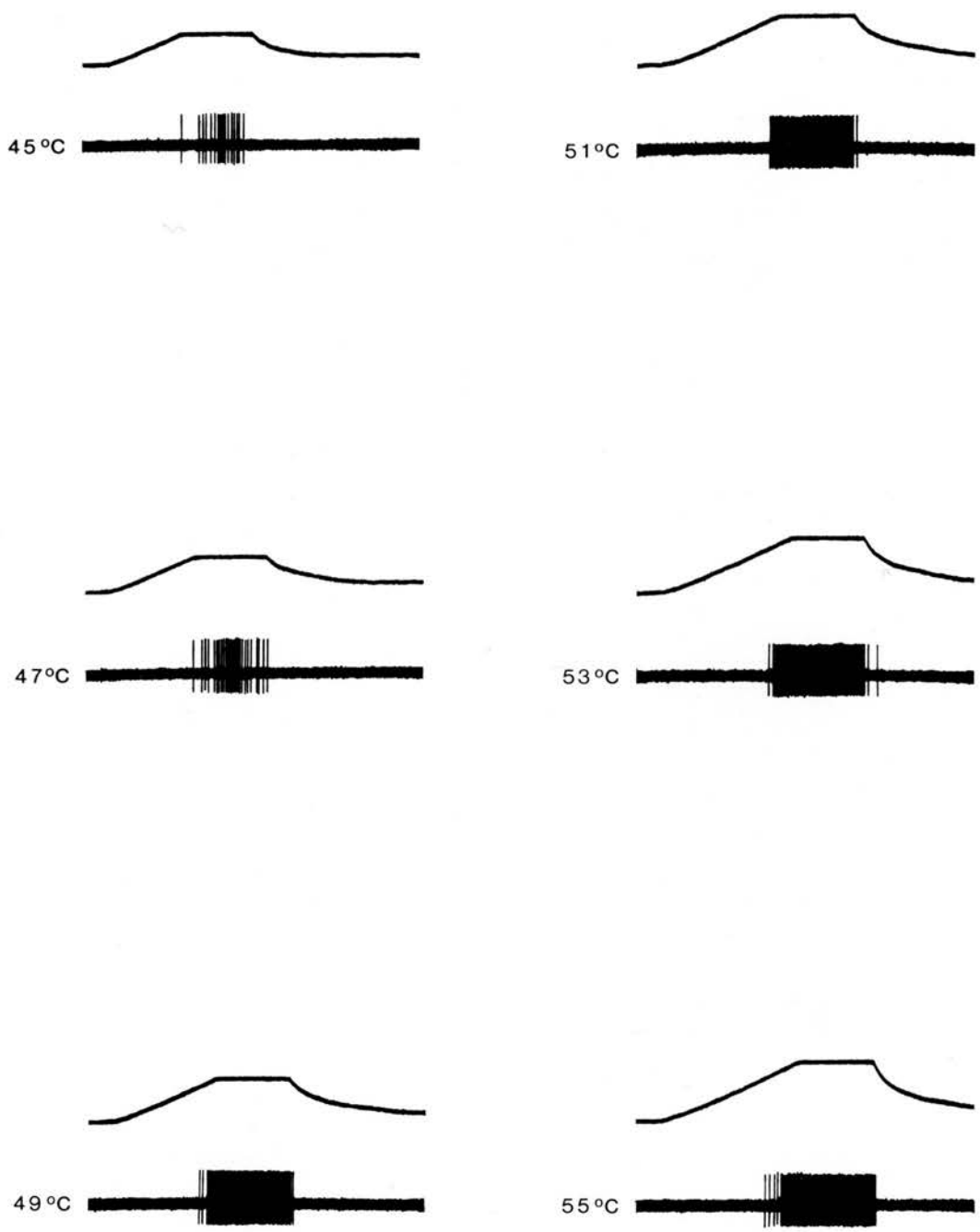

45°C
51°C
47°C
53°C
49°C
55°C

Fig. 3:2. B. The relationship between the receptive field hold temperature and the response of unit no. 56, measured as the number of impulses discharged during the 10 sec. hold. The response increased up to the highest temperature tested (59°C).

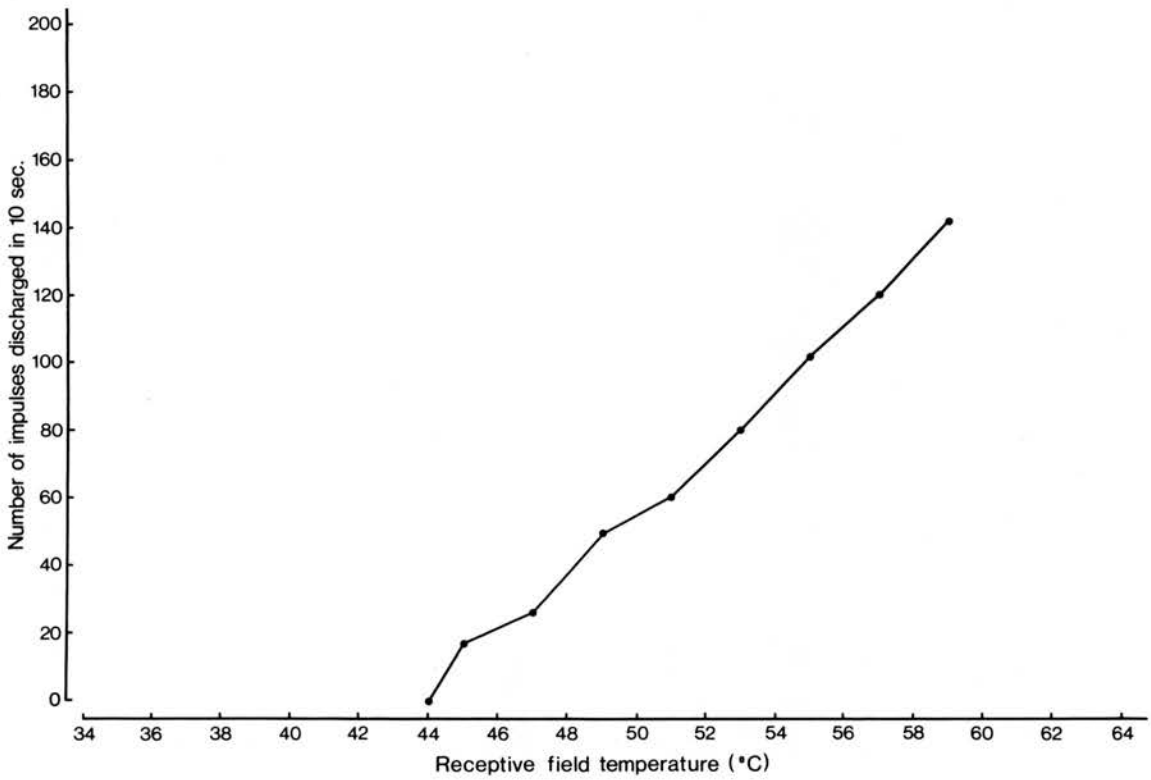

Number of impulses discharged in 10 sec.
200
180
160
140
120
100
80
60
40
20
0
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
Receptive field temperature (°C)

Fig. 3:2 C. The afferent discharges of a cutaneous nociceptor recorded from the alveolar mandibular nerve of a beak-trimmed chicken, at different beak temperatures.

Specimen records. In each record, the lower trace is the action potential discharge and the upper trace is the beak surface temperature measured with a thermocouple positioned on the centre of the receptive field. The temperature of the receptive field was first raised to 34 °C, then ramp and hold heat stimuli at different temperatures were delivered. The stimuli were applied in random sequence, with 3 minutes between successive stimuli. The hold temperature of each stimulus was maintained for 10 sec. The value of the hold temperature is indicated on the left of each record. Note the bursting pattern of the discharge.

Unit no. 43. The receptive field of this unit was located 3 mm. proximal to the tip of the trimmed beak. The receptive field was circular, 2 x 2 mm. Its mechanical threshold, measured with von Frey hairs, was 21g. This unit was recorded 7 days after beak amputation.

Calibration bar (time) = 10 sec.

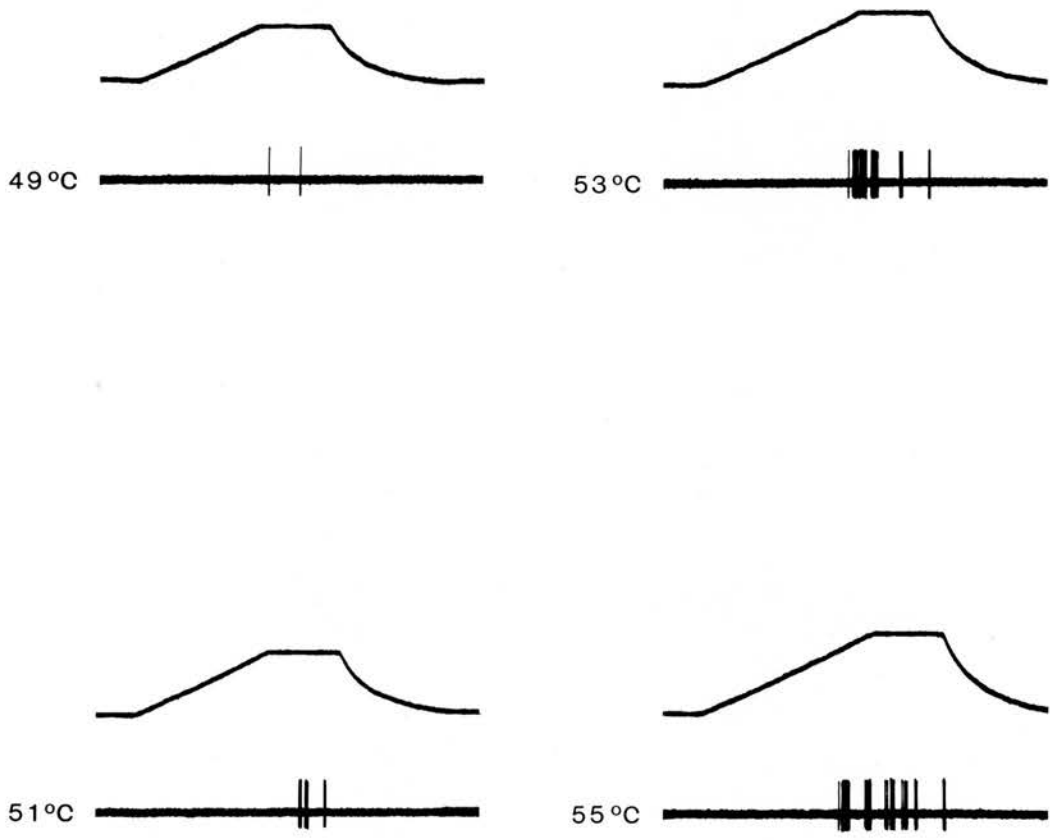

49°C
53°C
51°C
55°C

Fig. 3:2. D. The relationship between the receptive field hold temperature and the response of unit no. 43, measured as the number of impulses discharged during the 10 sec. hold. The peak response occurred at 53°C.

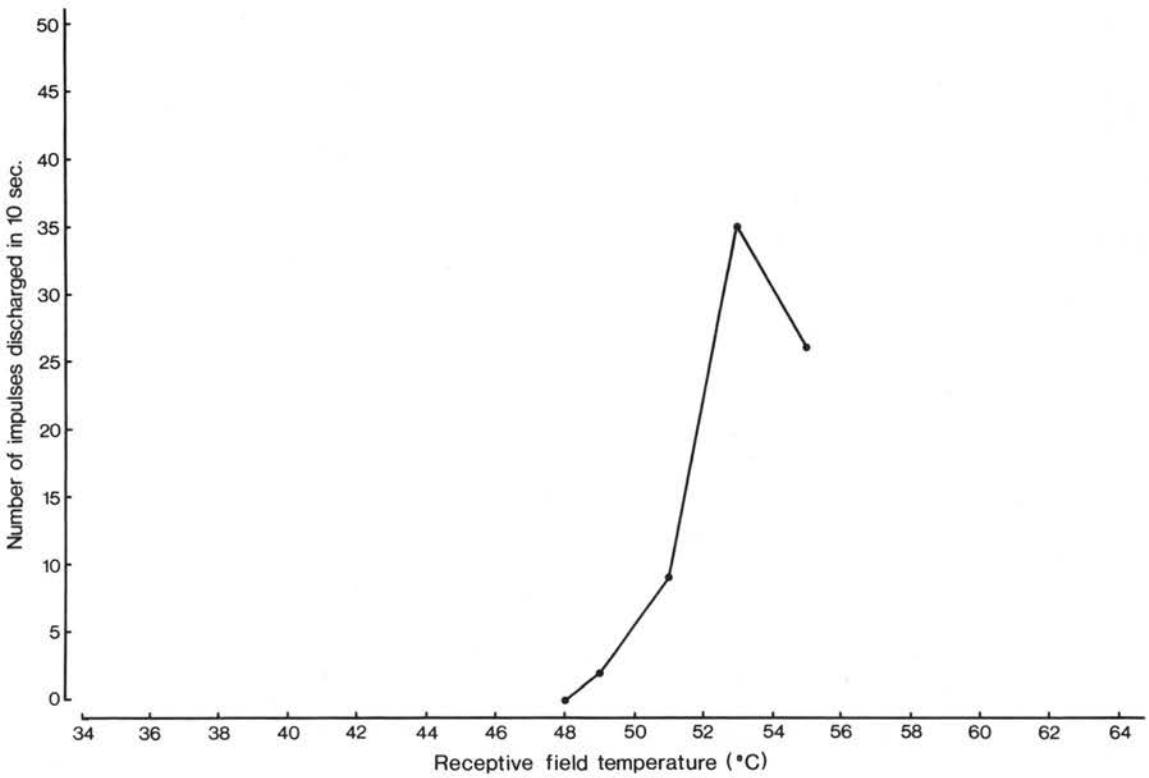
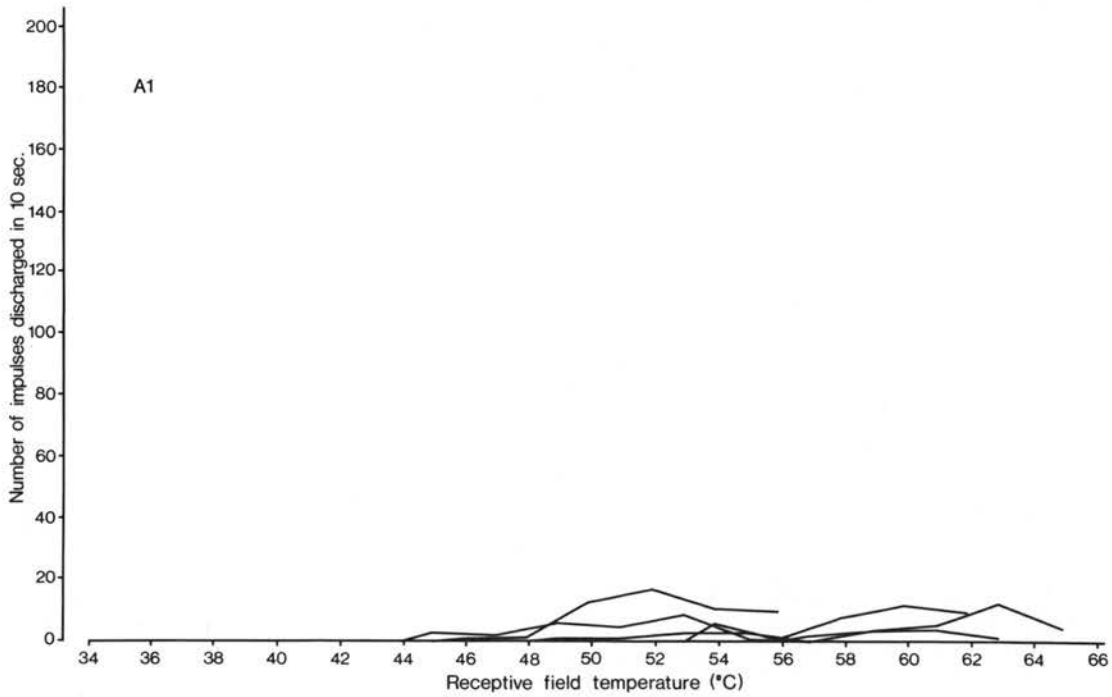
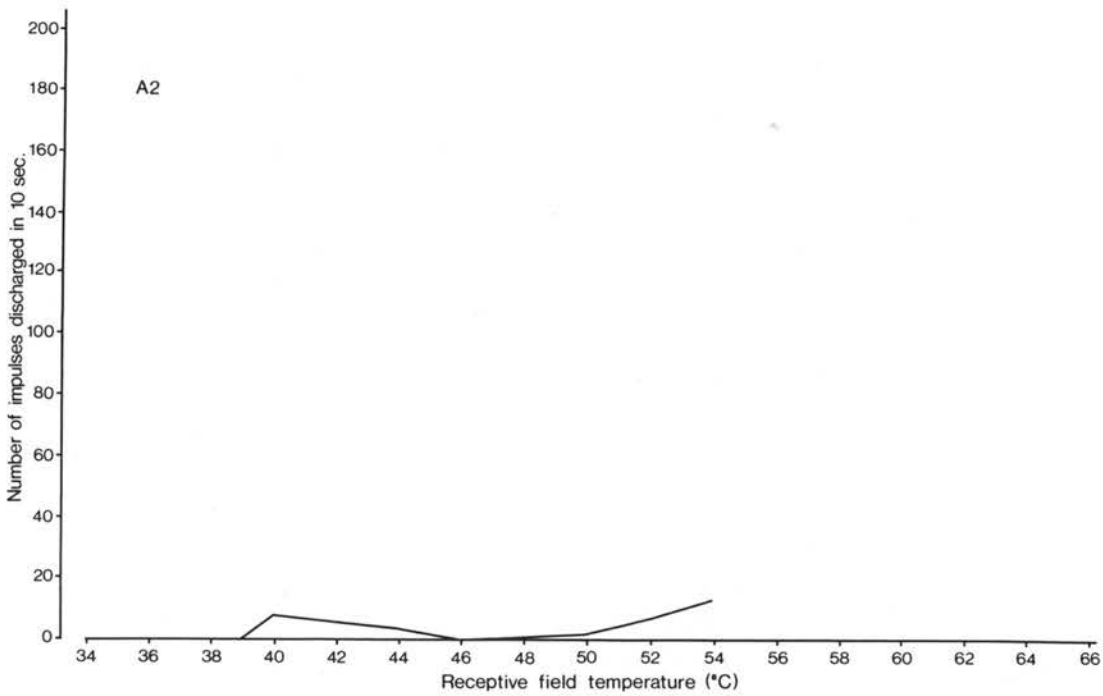
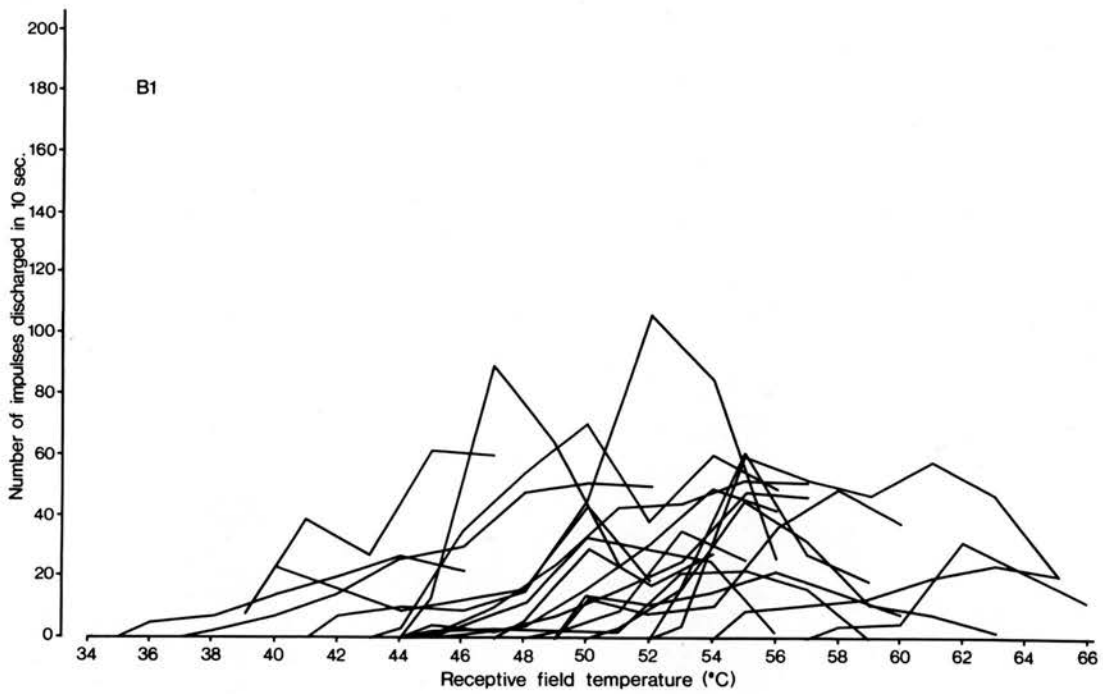
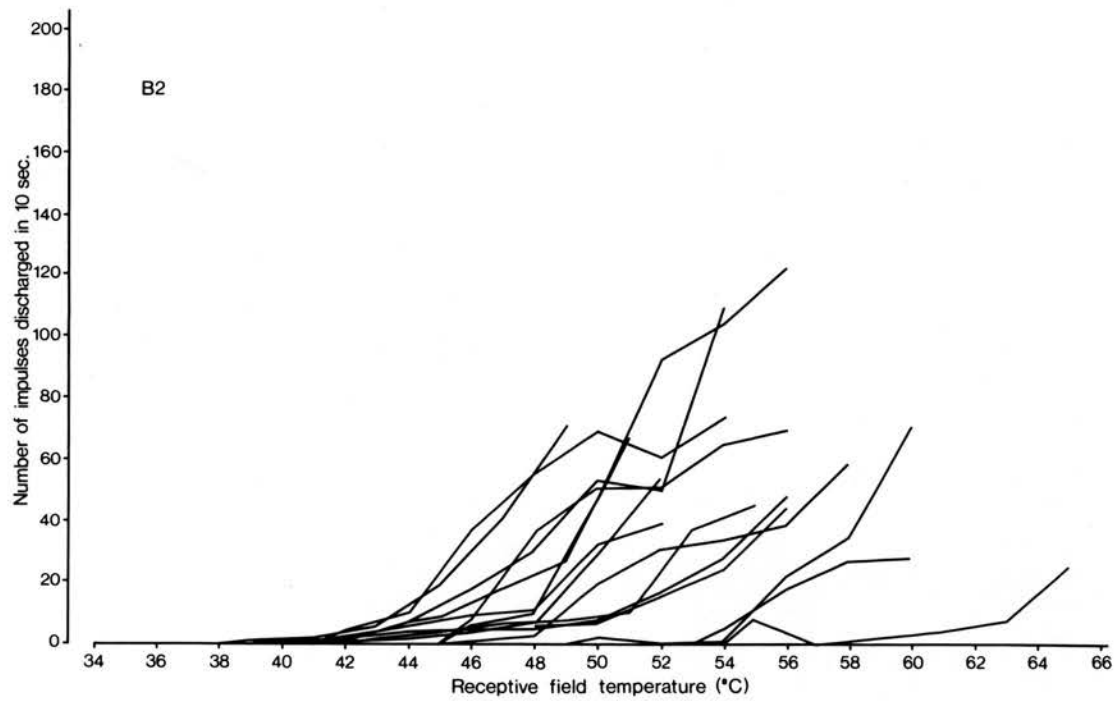

Number of impulses discharged in 10 sec.
0
5
10
15
20
25
30
35
40
45
50
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
Receptive field temperature (°C)

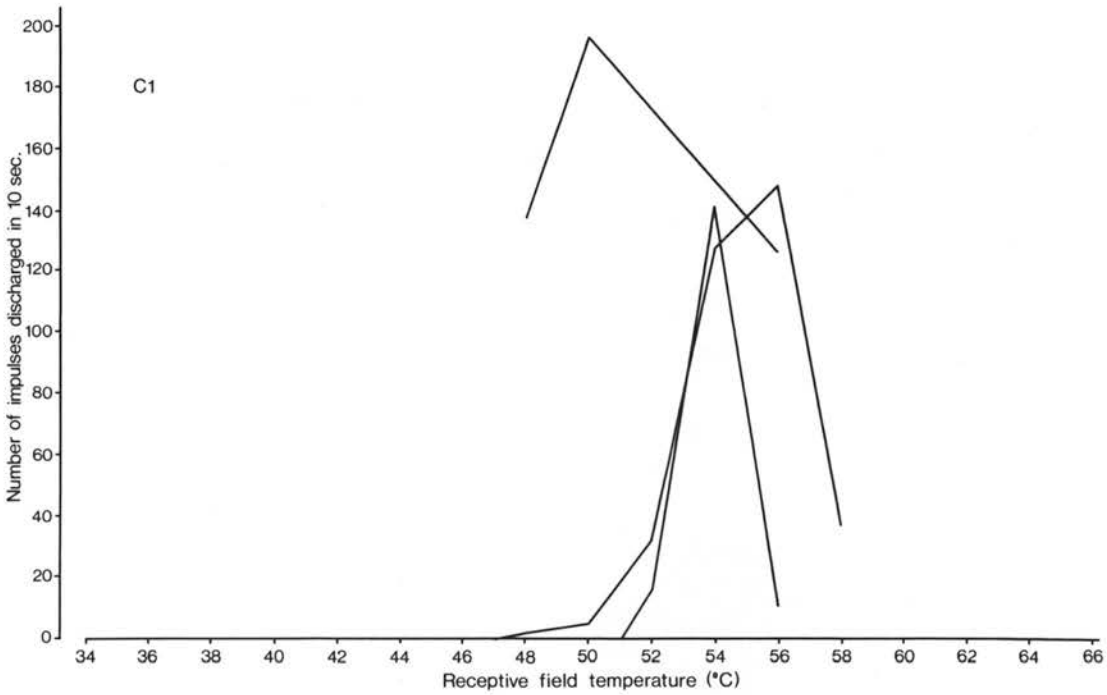
Fig. 3:3 The stimulus-response curves for all 47 heat-sensitive nociceptors recorded from beak-trimmed birds. For convenience of illustration, and for comparison with the stimulus-response curves obtained for the normal nociceptors, they are presented in six groups in a similar manner to fig. 2:3. Group A1 contains the units which had peak discharges of <20 impulses per 10sec (n=5), B1 units with peak discharges of 20 to 130 impulses per 10 sec (n=21), and C1 units with peak discharges of >140 impulses per 10 sec (n=3). Groups A2, B2 and C2 contain units which did not show a peak discharge within the range of temperatures tested. They are grouped according to the maximal discharge observed.


A1
Number of impulses discharged in 10 sec.
200
180
160
140
120
100
80
60
40
20
0
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
66
Receptive field temperature (°C)


A2
Number of impulses discharged in 10 sec.
200
180
160
140
120
100
80
60
40
20
0
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
66
Receptive field temperature (°C)


B1
Number of impulses discharged in 10 sec.
0
20
40
60
80
100
120
140
160
180
200
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
66
Receptive field temperature (°C)


B2
Number of impulses discharged in 10 sec.
200
180
160
140
120
100
80
60
40
20
0
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
66
Receptive field temperature (°C)


C1
Number of impulses discharged in 10 sec.
0
20
40
60
80
100
120
140
160
180
200
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
66
Receptive field temperature (°C)

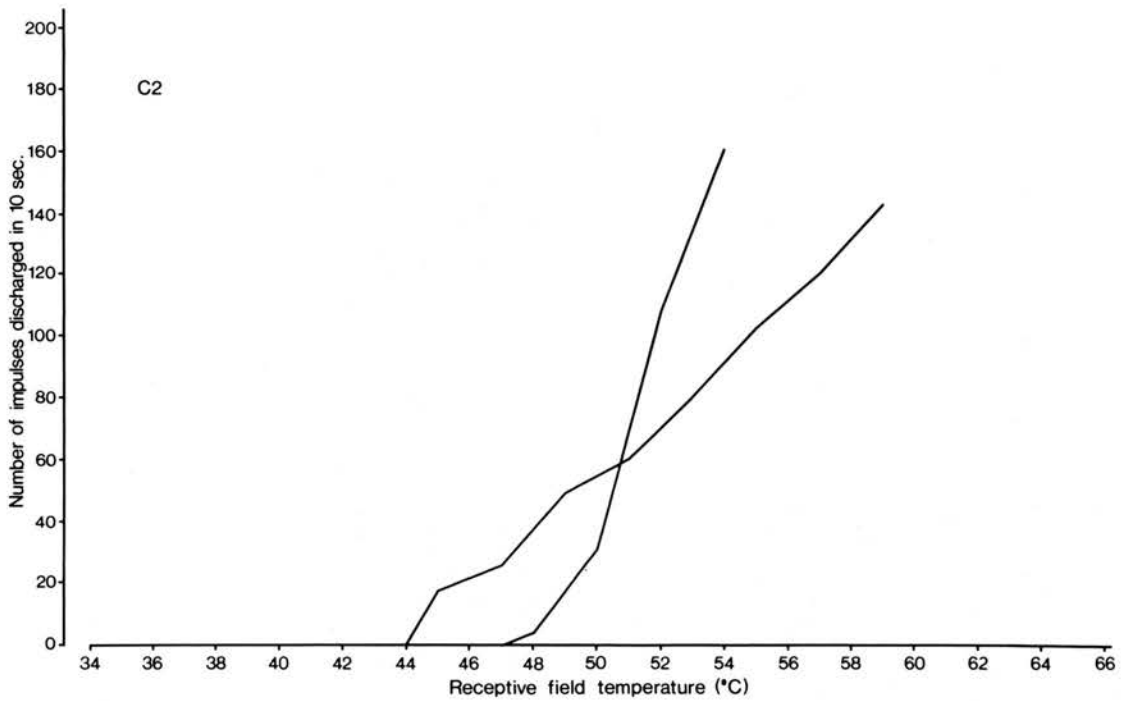

C2
Number of impulses discharged in 10 sec.
200
180
160
140
120
100
80
60
40
20
0
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
66
Receptive field temperature (°C)

Fig. 3:4. Distribution of the temperatures at which the heat sensitive nociceptors from beak trimmed birds displayed their peak or maximal response. Twenty-nine units showed a peak response within the range of temperatures tested (solid blocks). Eighteen units did not show a peak response within the range of temperatures tested and the highest temperatures tested on these units are included (open blocks).

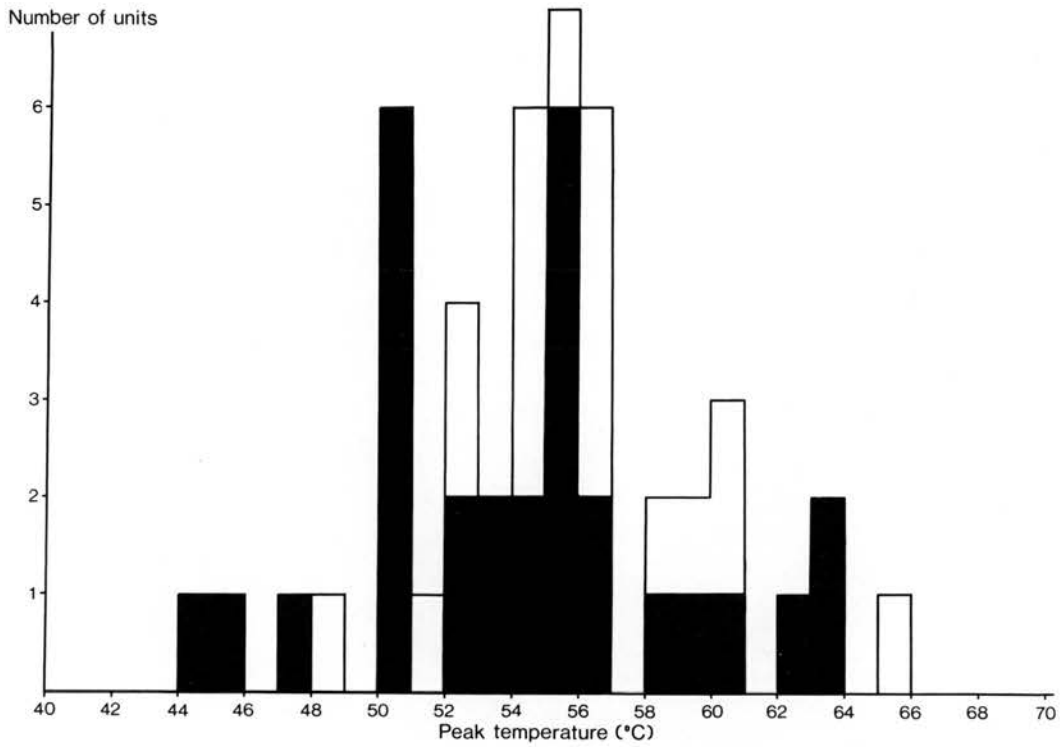

Number of units
6
5
4
3
2
1
40
42
44
46
48
50
52
54
56
58
60
62
64
66
68
70
Peak temperature (°C)

Fig. 3:5. Peak/maximal responses of the 47 heat-sensitive nociceptors recorded from beak-trimmed birds. Twenty nine units showed a peak response within the temperature range tested (solid blocks). Eighteen units did not show a peak response within the temperature range tested. The maximal responses obtained for these eighteen units are included (open blocks). The majority of the peak/maximal responses (n=34) were <80 impulses per 10 sec.

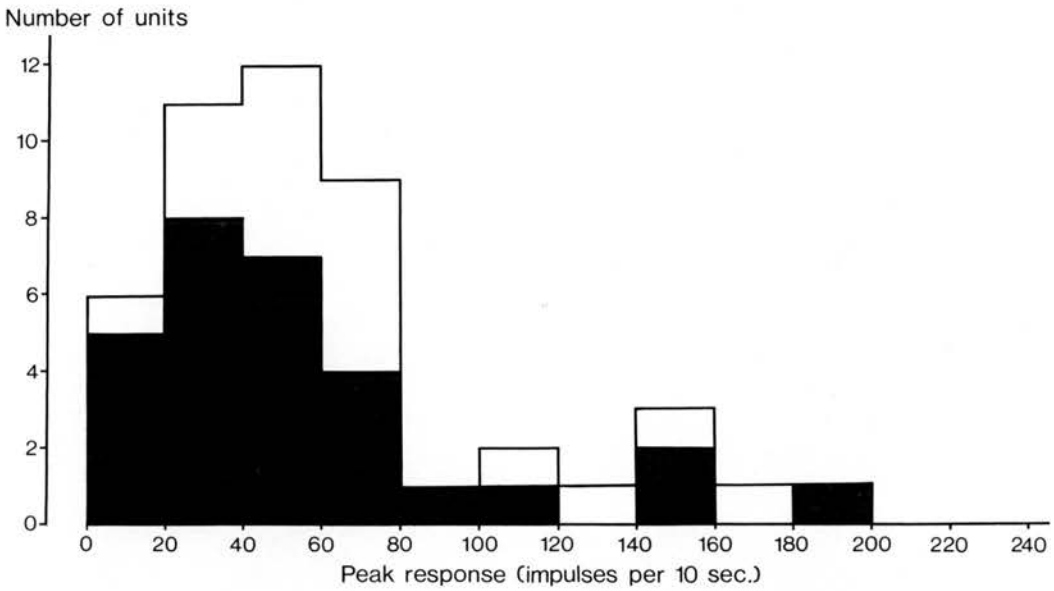
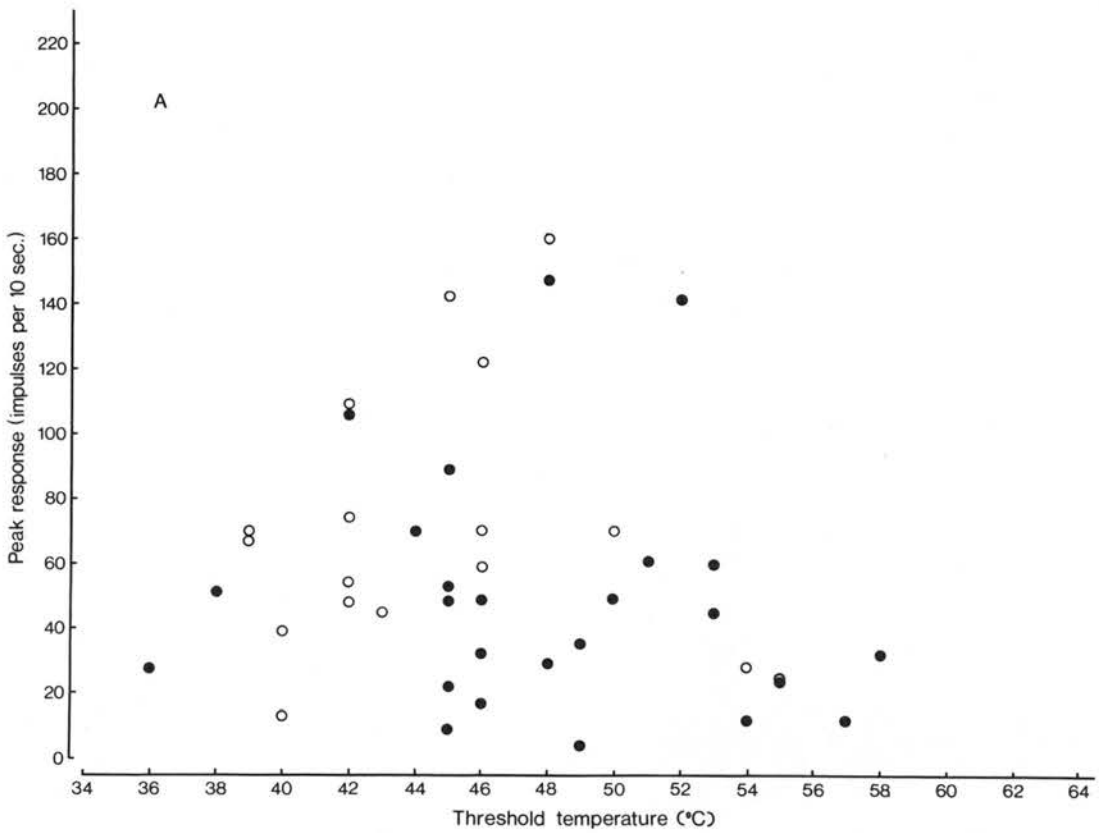

Number of units
12
10
8
6
4
2
0
0
20
40
60
80
100
120
140
160
180
200
220
240
Peak response (impulses per 10 sec.)

Fig. 3:6.
A. Illustrates the relationship between the peak/maximal response and the threshold temperature for the heat-sensitive nociceptors, recorded from beak trimmed birds. 42 units are shown here. Threshold measurements were not obtained for the remaining 5 units.

B. Illustrates the relationship between the peak/maximal response and the peak/maximal temperature for the 47 heat sensitive nociceptors.

● Represents one unit of the 29 showing a peak response.
o Represents one unit of the 18 for which a maximal response only was obtained.

Note that no simple correlation can be detected in either case.


A
Peak response (impulses per 10 sec.)
0
20
40
60
80
100
120
140
160
180
200
220
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
Threshold temperature (°C)

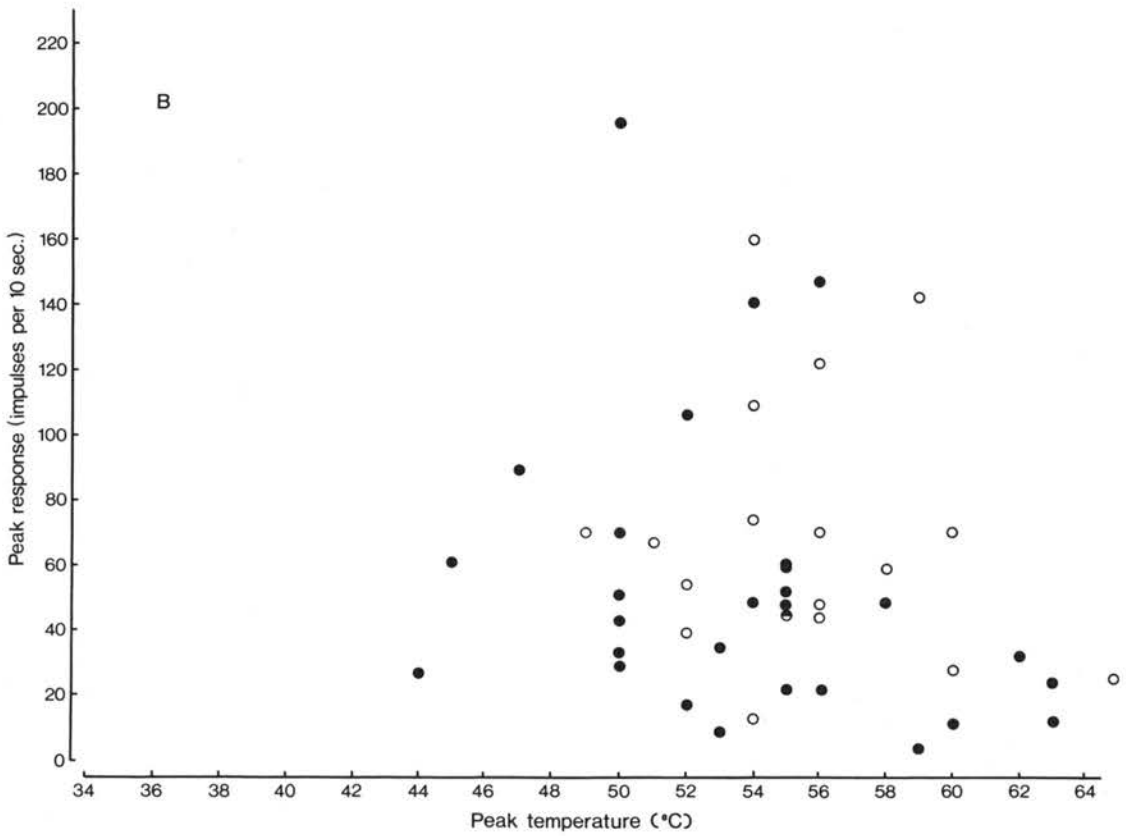

B
Peak response (impulses per 10 sec.)
220
200
180
160
140
120
100
80
60
40
20
0
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
Peak temperature (°C)

Fig. 3:7 The relationship between the threshold and the peak/maximal temperatures for the heat sensitive nociceptors recorded from beak-trimmed birds. As in fig.3:6A, 42 units are shown here.

Symbols used are the same as in fig 3:6, with the addition of : ◉ which represents 3 units of the 29 showing a peak response and ○ , which represents 2 units of the 18 for which maximal response only was obtained. ◓ = ● + ○

Note that there is evidence here for a direct relationship between the threshold and the temperature necessary to provoke the peak/maximal response.

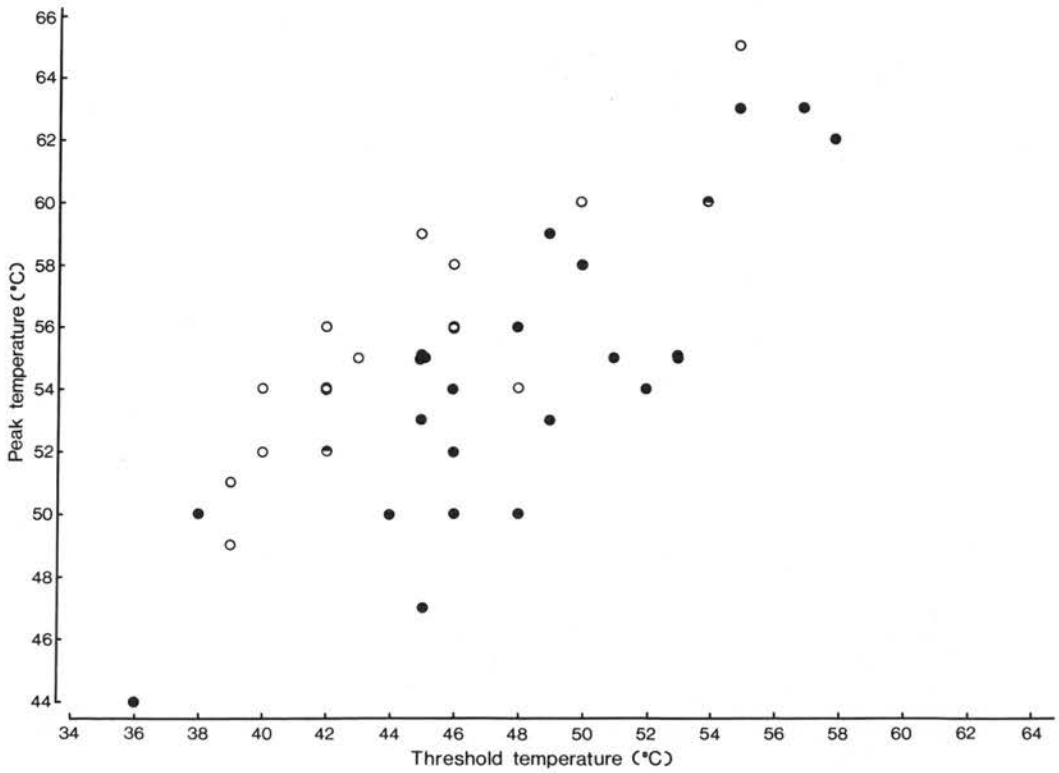

Peak temperature (°C)
66
64
62
60
58
56
54
52
50
48
46
44
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
Threshold temperature (°C)

Fig. 3:8 The stimulus-response curves for the 47 heat sensitive nociceptors recorded from beak-trimmed birds. The portion of each curve from threshold to peak/maximal response is shown. The stimulus temperature values have been normalized, to facilitate a visual comparison of the thermal ranges and sensitivities of the units. Normalization was carried out with respect to threshold, with Tt representing threshold, Tt+2 2 °C above threshold, Tt+4 4 °C above threshold, etc..

The stimulus-response curves have been divided into six groups in the same manner as shown in fig. 3:3. Thus group A1 contains the units showing peak discharges of <20 impulses per 10 sec; group A2 contains the units showing maximal discharges of <20 impulses per 10 sec.. Group B1 contains the units showing peak discharges of 20 to 130 impulses per 10 sec; group B2 contains the units showing maximal discharges of 20 to 130 impulses per 10 sec. Group C1 contains the units showing peak discharges of >140 impulses per 10sec; group C2 contains the units showing maximal discharges of >140 impulses per 10 sec.

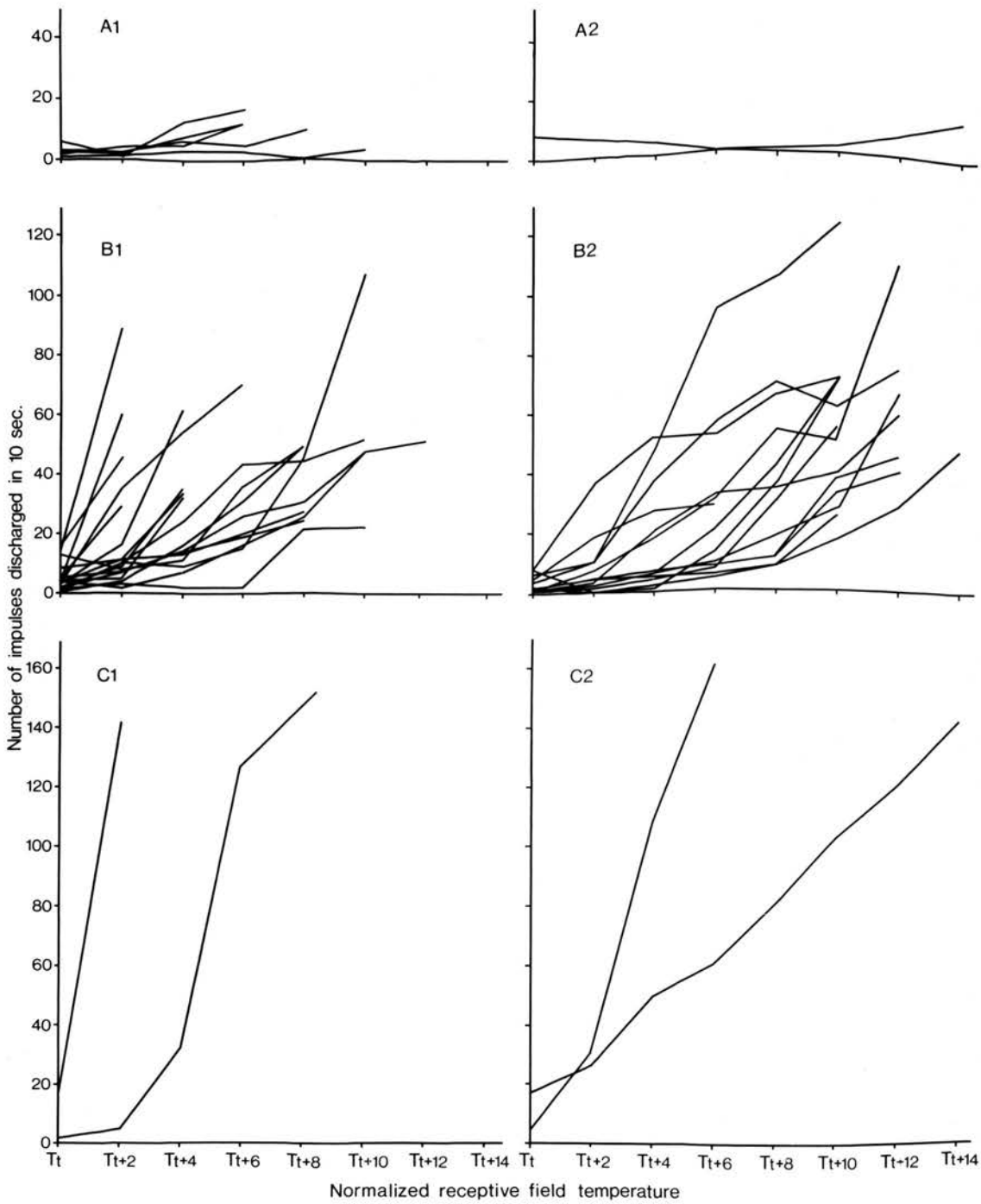

A1
A2
B1
B2
C1
C2
Number of impulses discharged in 10 sec.
Normalized receptive field temperature
Tt
Tt+2
Tt+4
Tt+6
Tt+8
Tt+10
Tt+12
Tt+14

Fig. 3:9 Reproducibility of the stimulus-response curves, illustrated by the responses of four heat sensitive nociceptors to repeated testing at each stimulus temperature.

● Indicates the responses to the first series of stimuli, o to the second series and ▲ to the third series of stimuli. Within each stimulus series the suprathreshold stimuli were delivered in random order. The time interval between stimuli and between each series was 3 minutes.

Some characteristics of these units are tabulated below:

Unit no.	Time at which recorded (post-beak trimming) (days)	Receptive field location (mm from beak tip)	Mechanical Threshold (g.)
58	53	2	4
60	64	12	12
61	64	8	12
70	84	0	12

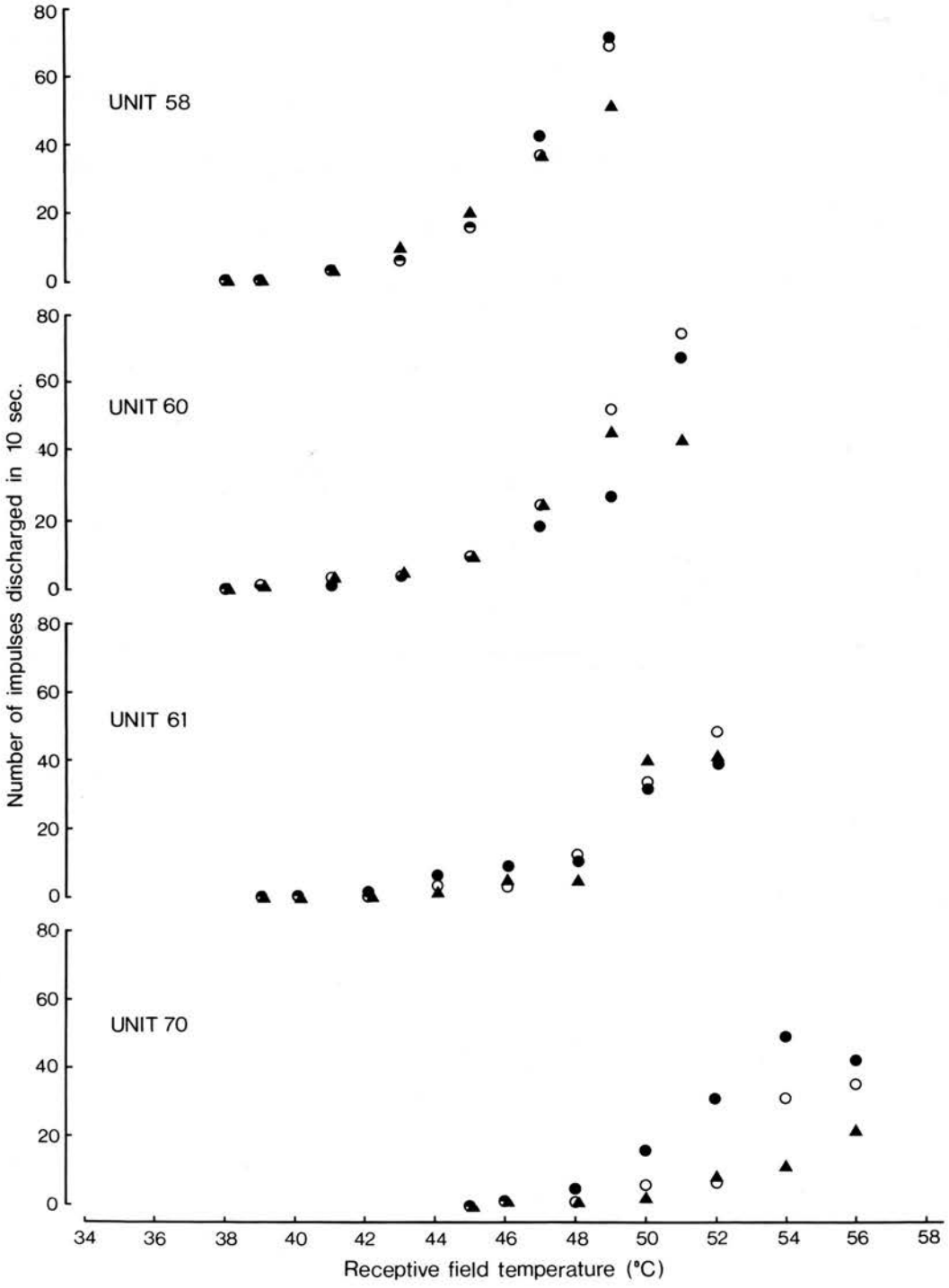

UNIT 58
UNIT 60
UNIT 61
UNIT 70
Number of impulses discharged in 10 sec.
Receptive field temperature (°C)
0
20
40
60
80
34
36
38
40
42
44
46
48
50
52
54
56
58

Fig. 3:10. The stimulus-response relationship for unit 58. Each data point represents the arithmetic mean of the three seperate response values illustrated in Fig. 3:9. The data are shown plotted out on linear axes (A) untransformed, (B) after log transformation of the stimulus and (C) after log transformation of both stimulus and response.

The figure provides a visual impression of goodness of fit of the data, i.e. the nearest approximation to a straight line relationship, to different intensity functions. The three intensity functions tested here are, after transformation to linear axes,

(A) Linear, $R=b.S + A$
(B) Logarithmic, $R=b.\log S + A$
(C) Power, $\log R=b.\log S + \log A$

where R= response magnitude
b= slope
S= stimulus intensity
A= intercept

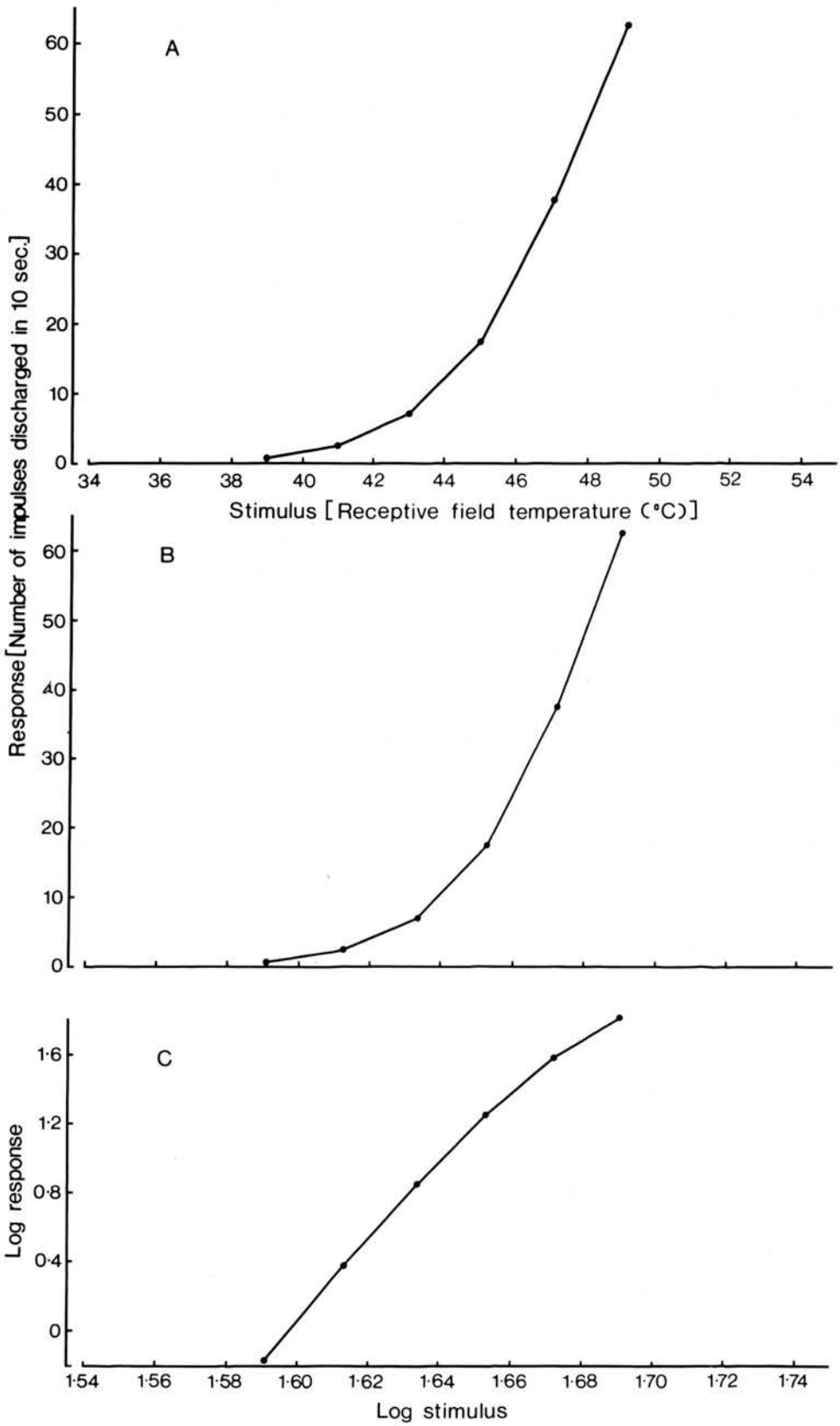

A
B
C
Response [Number of impulses discharged in 10 sec.]
Stimulus [Receptive field temperature (°C)]
Log response
Log stimulus
0
10
20
30
40
50
60
34
36
38
40
42
44
46
48
50
52
54
0
0·4
0·8
1·2
1·6
1·54
1·56
1·58
1·60
1·62
1·64
1·66
1·68
1·70
1·72
1·74

Fig. 3:11 The stimulus-response data of unit 58 plotted out in the same manner as in Fig. 3:10 after normalizing the stimulus temperature values. The normalization was carried out with respect to threshold, by assigning the threshold temperature (Tt) the numerical value of 1, thus Tt=1, Tt+2=3, Tt+4=5, etc.. The three intensity functions tested, after normalization and transformation to linear axes are:

(A) Linear, $R=b.(S-S_o) + A$
(B) Logarithmic, $R=b.\log(S-S_o) + A$
(C) Power, $\log R=b.\log(S-S_o) + \log A$

where S_o= highest subthreshold stimulus intensity, i.e. 1°C below threshold.

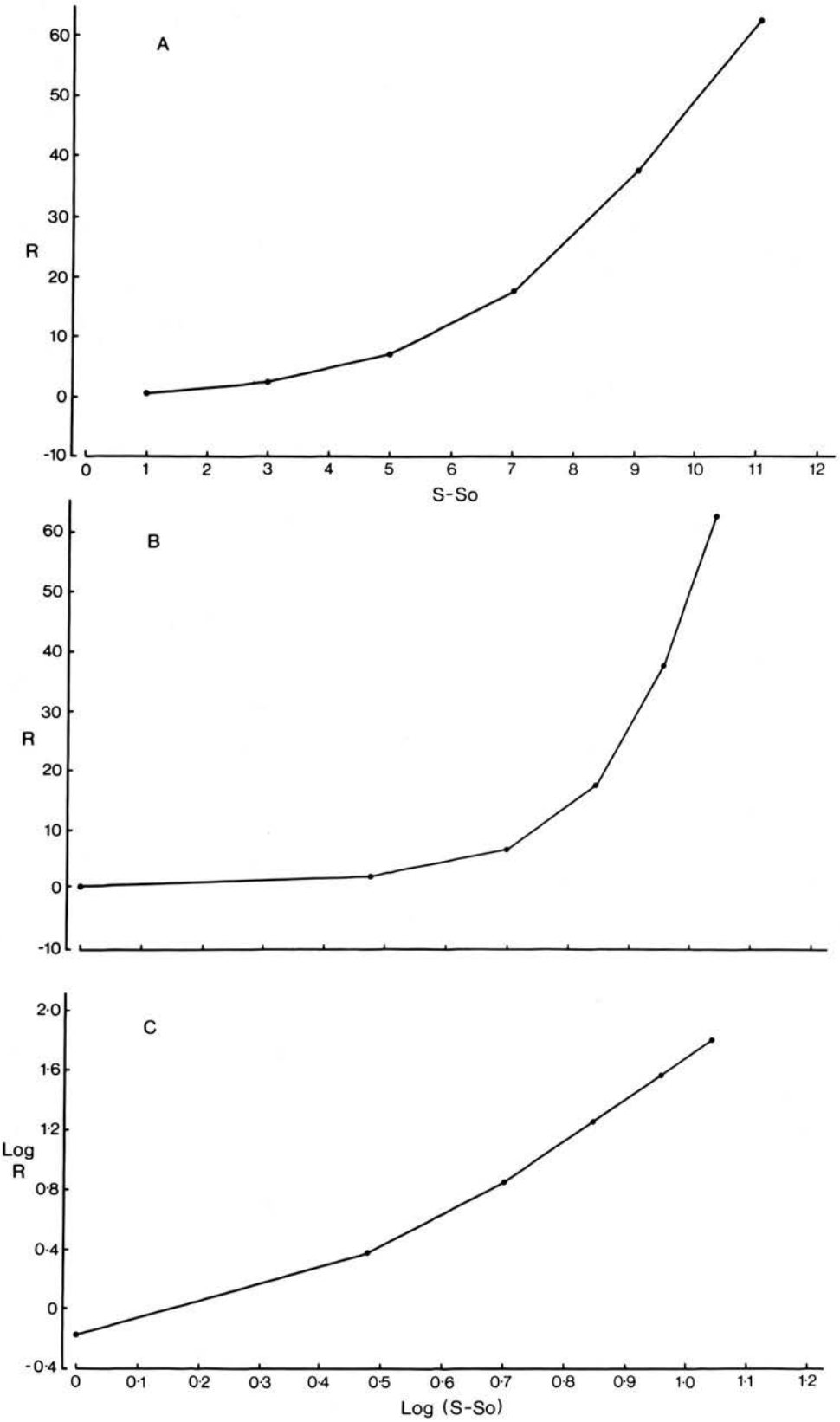

A
R
S-So
B
R
C
Log
R
Log (S-So)

Fig 3:12. The stimulus-response data of unit 58 plotted out in the same manner as Fig. 3:11, with the calculated regression lines fitted through the data points. The correlation coefficients for the regression lines are, respectively,

(A) Linear, 0.9338
(B) Logarithmic, 0.7778
(C) Power, 0.9779

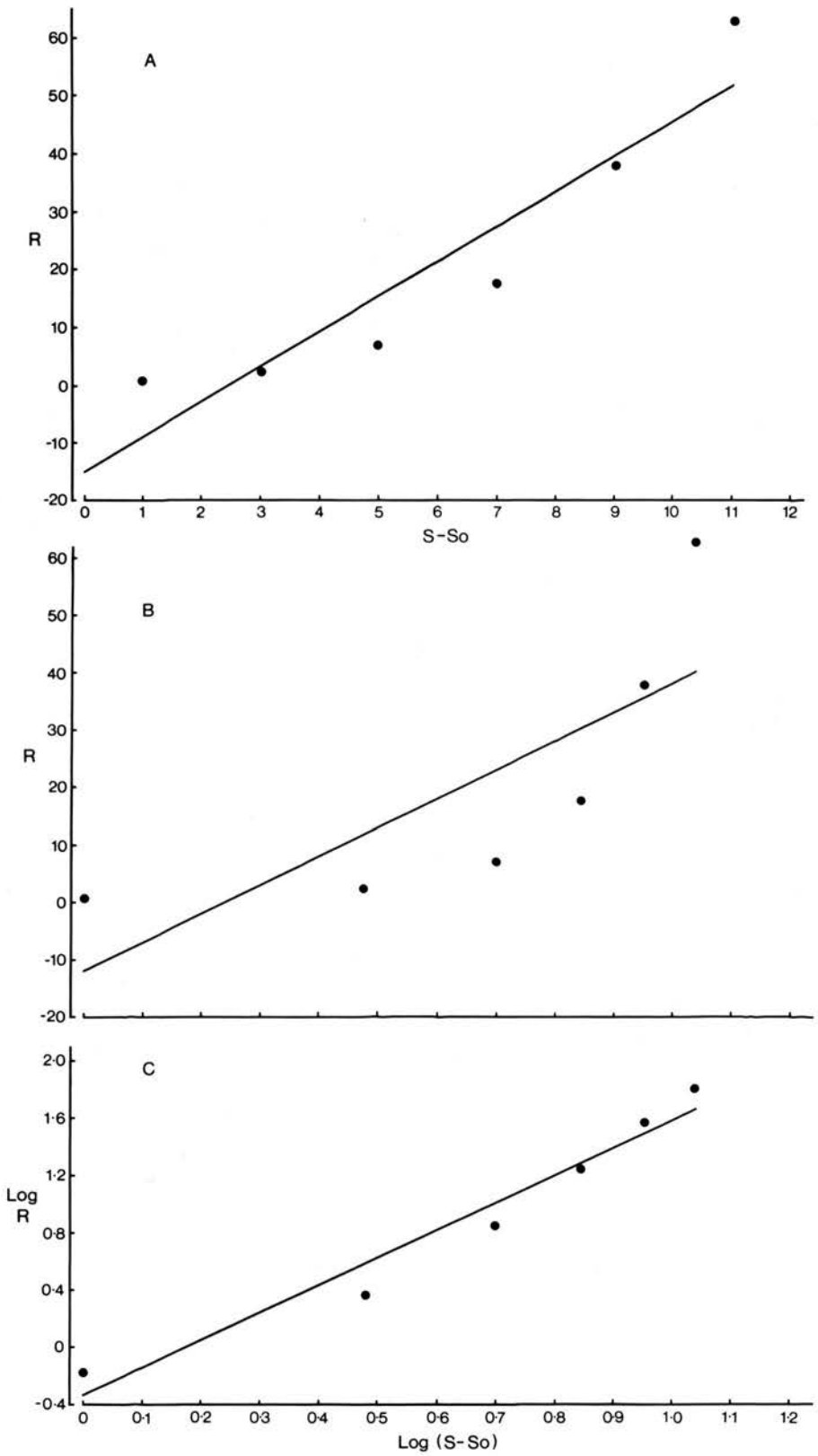

A
R
S-So
B
R
C
Log
R
Log (S-So)

Fig. 3:13. The sensitivities of the heat-sensitive nociceptors recorded from the beak-trimmed birds. The values for the sensitivities were derived from the regression lines fitted to the power-transformed stimulus-response data. Solid blocks represent the units which showed a peak response within the range of temperatures tested (n=25). Open blocks represent the units which did not show a peak response within the range of temperatures tested (n=17). Note that 5 units , out of the total sample of 47 units, are not represented here. Threshold measurements were not obtained for these 5 units,, precluding regression analysis comparable with that carried out on the other 42 units.

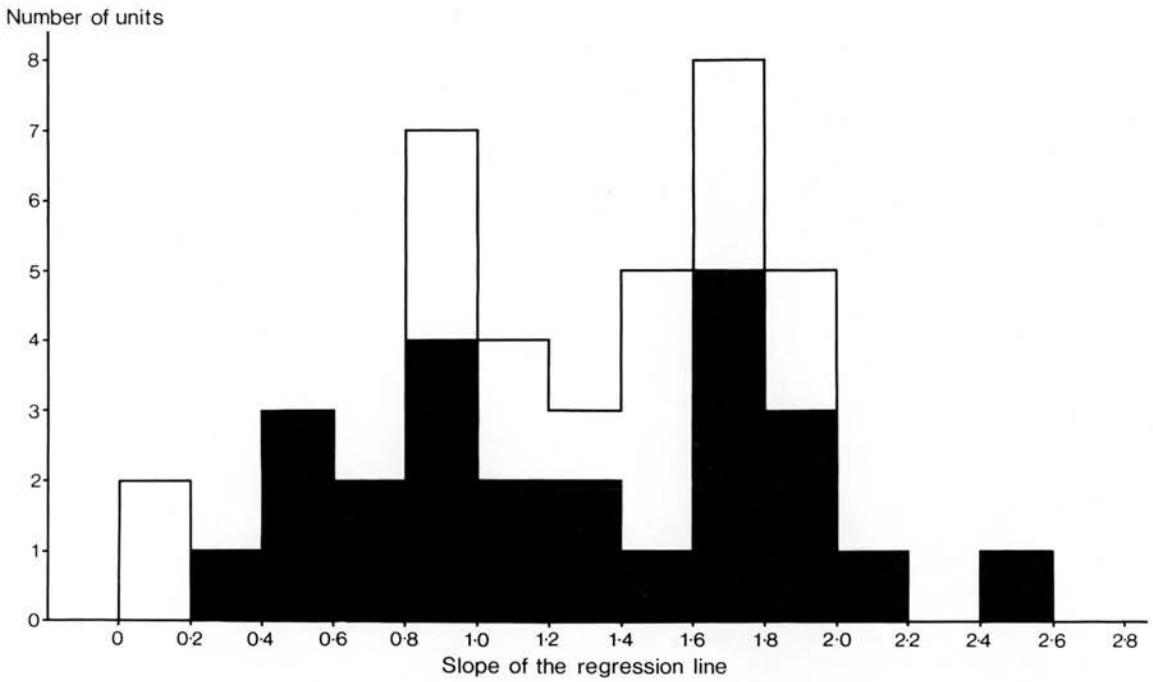

Number of units
8
7
6
5
4
3
2
1
0
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
1·8
2·0
2·2
2·4
2·6
2·8
Slope of the regression line

Table 3:1. The raw data from which the stimulus response curves illustrated in Fig. 3:3 were constructed. The values in the body of the table are the numbers of impulses discharged during the 10 sec. hold phase of a ramp and hold heat stimulus. The temperatures of the stimuli are displayed on the top of the table. Each row contains the stimulus-response data for one unit.

NUMBER OF IMPULSES DISCHARGED IN 10 SECONDS

Unit Number	Temperature (°C)																											
	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
37							7		10		9		15		45		106		85		26										
38									3		35		54		70		38		60		49										
39																				9		11		13		20		24		21	
40																				8		0		2		4		8		25	
41											6		10		47		92		104		122										
42																							4		5		32		22		12
43														2		9		35		26										
44										1		10		24		43		44		52		51								
45										2		3		2		2		21		22		16		0						
46													4		31		108		160											
47										3		2		6		5		9		1		0								
48							2		4		5		5		8		17		28		48										
49											8		36		51		51		65		70										
50														1		1		3		3		0		4		4		1			
51																		16		45		32		11		8		2			
52													2		5		32		127		147		37								
53											4		11		33		29		25		2										
54							4		10		37		55		69		61		74												
55					8		6		4		0		1		2		7		13												
56										17		26		49		60		80		102		120		142						
57													137		196						126										
58				1		2		6		19		41		70																	
59										13		89		64		24															
60				1		1		4		9		18		27		67															
61					1		2		6		9		11		32		39														
62															2		0		1		22		35		70						
63											1		1		13		17		11		10										
64																	16		141		11										
65																						2		4		5		12		4	
66																		4		60		52		47		58		47		21	
67																			5		18		27		28						
68					23				9				16		43		19														
69																3		16		61		28		19						
70											1		4		16		31		49		42										
71															14		11		15		22		15		8						
72								2		4		7		8		11		37		45											
73							1		7		18		30		53		50		109												
74				8		39		27		61		60																			
75																			6		1		8		12		10				
76											1		3		20		31		34		39		59								
77													6		7		16		24		44										
78													5		29		17		28												
79							1		2		4		7		29		54														
80															13		8		11		36		49		38						
81										4		2		7		16		25		48		46									
82	5		7		14		20		27		22																				
83			2		7		14		26		30		48		51		50														

Table 3:2 Stimulus-response characteristics of 29 units which exhibited a peak response within the range of temperature tested.

- indicates no measurement was made.

UNIT	THRESHOLD TEMPERATURE, Tt(°C)	PEAK TEMPERATURE, Tp(°C)	TEMPERATURE RANGE, Tp-Tc (°C)	PEAK RESPONSE (IMPULSES/10 sec.)
37	42	52	10	106
38	44	50	6	70
39	55	63	8	24
42	58	62	4	32
43	49	53	4	35
44	45	55	10	52
45	45	55	10	22
47	45	53	8	9
50	49	59	10	4
51	53	55	2	45
52	48	56	8	147
53	46	50	4	33
57	-	50	-	196
59	45	47	2	89
63	46	52	6	17
64	52	54	2	141
65	57	63	6	12
66	53	55	2	60
68	-	50	-	43
69	51	55	4	61
70	46	54	8	49
71	-	56	-	22
74	-	45	-	61
75	54	60	6	12
78	48	50	2	29
80	50	58	8	49
81	45	55	10	48
82	36	44	8	27
83	38	50	12	51

Table 3:3 Stimulus-response characteristics of the 18 units which did not exhibit a peak response within the range of heat stimuli tested. These 18 units displayed an increasing response up to the highest temperature tested.

Unit	Threshold Temperature Tt(°C)	Highest Temperature Th(°C)	Temperature Range Th-Tt(°C)	Maximal Response Impulses/10 sec.
40	55	65	10	25
41	46	56	10	122
46	48	54	6	160
48	42	56	14	48
49	46	56	10	70
54	42	54	12	74
55	40	54	14	13
56	45	59	14	142
58	39	49	10	70
60	39	51	12	67
61	40	52	12	39
62	50	60	10	70
67	54	60	6	28
72	43	55	12	45
73	42	54	12	109
76	46	58	12	59
77	-	56	-	44
79	42	52	10	54

- indicates no measurement was made

Table 3:4 Summary of the stimulus-response characteristics from tables 3:2 and 3:3.

a)

Units Tested To Peak Response	Threshold Temperature, Tt(°C)	Peak Temperature Tp(°C)	Temprature Range Tp-Tt(°C)	Peak Response Impulses/10sec
n	25	29	25	29
Range	36-58	44-63	2-12	4-196
Median	48	54	6	45
Mean	48.00	53.83	6.40	53.31
Standard Error	1.08	0.90	0.62	8.30

b)

Units Not Tested To Peak Response	Threshold Temperature Tt(°C)	Highest Temperature Tested Th(°C)	Temperature Range Th-Tt(°C)	Maximal Response Impulses/10sec
n	17	18	17	18
Range	39-55	49-65	6-14	13-160
Median	43	56	12	67
Mean	44.65	55.61	10.94	68.83
Standard Error	1.18	0.90	0.57	9.51

c)

All Units	Threshold Temperature Tt(°C)	Highest Temperature Tested Th(°C)	Temperature Range Th-Tt(°C)	Maximal Response Impulses/10sec
n	42	47	42	47
Range	36-58	44-65	2-14	4-196
Median	46	55	10	49
Mean	44.64	54.51	8.24	59.26
Standard Error	0.83	0.66	0.55	6.32

Table 3:5 Statistical parameters from the regression analysis of the stimulus-response relationship for 4 nociceptor units (58,60,61 and 70).

The intensity functions listed are those described in the text, i.e.;

Linear, $R = b.(S-So)+A$

Logarithmic, $R = b.\log(S-So)+A$

Power, $R = A.(S-So)^{b}$

r = correlation coefficient
b = slope of the regression line
A = intercept
P = significance level for r
NS = no significant difference from zero correlation (P>0.05)

Unit	Intensity Function	r	b	A	P(<)
58	Linear	0.9338	6.10	-15.24	0.01
	Logarithmic	0.7778	49.50	-11.80	0.05
	Power	0.9779	1.91	-0.34	0.001
60	Linear	0.9207	4.92	-14.27	0.01
	Logarithmic	0.7472	44.43	-12.37	0.05
	Power	0.9298	1.52	-0.14	0.001
61	Linear	0.8897	3.57	-11.29	0.01
	Logarithmic	0.7162	31.97	-9.72	0.05
	Power	0.9512	1.96	-0.76	0.001
70	Linear	0.9663	3.58	-6.60	0.001
	Logarithmic	0.8518	30.78	-5.72	0.02
	Power	0.9659	1.58	-0.16	0.001

Table 3:6. Statistical parameters of the regression analysis of the stimulus response relationship for the units which displayed a peak response within the range of heat stimuli tested. Symbols used in the table are the same as those used in table 3:5.

Unit	Intensity Function	r	b	A	P(<)
	Linear	0.8324	8.66	-19.94	0.05
37	Logarithmic	0.6499	65.91	-12.12	NS
	Power	0.7860	0.95	0.64	0.05
	Linear	0.9860	11.00	-3.50	0.01
38	Logarithmic	0.9948	77.59	1.29	0.001
	Power	0.9752	1.66	0.56	0.01
	Linear	0.9714	1.95	5.65	0.01
39	Logarithmic	0.8674	14.58	6.72	0.05
	Power	0.9144	0.43	0.90	0.02
	Linear	0.8813	7.00	-7.33	NS
42	Logarithmic	0.7649	34.02	0.33	NS
	Power	0.8059	1.12	0.50	NS
	Linear	0.9489	8.25	-9.42	NS
43	Logarithmic	0.8634	42.03	-1.15	NS
	Power	0.9841	1.71	0.26	0.02
	Linear	0.9760	5.37	-3.23	0.001
44	Logarithmic	0.9501	50.98	-5.13	0.01
	Power	0.9848	1.69	0.10	0.001
	Linear	0.8270	2.2	-4.53	0.05
45	Logarithmic	0.6627	17.19	-2.84	NS
	Power	0.6527	0.88	0.09	NS
	Linear	0.8660	0.75	1.25	0.05
47	Logarithmic	0.7457	5.41	1.78	NS
	Power	0.7204	0.49	0.35	NS
	Linear	0.4140	0.17	0.97	NS
50	Logarithmic	0.4176	1.69	0.87	NS
	Power	0.8893	0.64	-0.08	0.02
	Linear	1.0000	14.50	1.50	0.001
51	Logarithmic	1.0000	60.78	16.00	0.001
	Power	1.0000	0.94	1.20	0.001
	Linear	0.9403	20.60	-40.40	0.01
52	Logarithmic	0.8228	150.95	-27.23	0.05
	Power	0.9516	2.10	0.10	0.01
	Linear	0.9582	7.25	-5.75	0.05
53	Logarithmic	0.8785	37.22	1.41	NS
	Power	0.9733	1.25	0.56	0.05

59	Linear	1.0000	38.00	-25.00	0.001
	Logarithmic	1.0000	159.29	13.00	0.001
	Power	1.0000	1.75	1.11	0.001
63	Linear	0.9393	3.00	-4.00	0.02
	Logarithmic	0.8495	18.97	-1.59	NS
	Power	0.8433	1.55	-0.20	NS
64	Linear	1.0000	62.50	-46.50	0.001
	Logarithmic	1.0000	261.99	16.00	0.001
	Power	1.0000	1.98	1.20	0.001
65	Linear	0.9202	1.55	-0.45	0.05
	Logarithmic	0.8141	9.59	0.91	NS
	Power	0.9366	0.81	0.26	0.02
66	Linear	1.0000	28.00	-24.00	0.001
	Logarithmic	1.0000	117.37	4.00	0.001
	Power	1.0000	2.47	0.60	0.001
69	Linear	0.9528	14.50	-16.83	0.05
	Logarithmic	0.8697	74.11	-2.39	NS
	Power	0.9897	1.82	0.44	0.02
70	Linear	0.9740	6.15	-10.55	0.001
	Logarithmic	0.8602	45.49	-6.87	0.05
	Power	0.9886	1.81	-0.08	0.001
75	Linear	0.7057	1.25	1.75	NS
	Logarithmic	0.5067	6.28	3.58	NS
	Power	0.2812	0.36	0.51	NS
78	Linear	1.0000	12.00	-7.00	0.001
	Logarithmic	1.0000	50.30	5.00	0.001
	Power	1.0000	1.60	0.70	0.001
80	Linear	0.8726	5.00	-1.60	0.05
	Logarithmic	0.6965	33.42	3.51	NS
	Power	0.6473	0.59	0.91	NS
81	Linear	0.9136	4.26	-8.54	0.01
	Logarithmic	0.7451	33.85	-5.66	NS
	Power	0.8057	1.09	0.28	0.05
82	Linear	0.9875	2.85	0.35	0.001
	Logarithmic	0.8959	21.66	1.71	0.02
	Power	0.9550	0.78	0.62	0.01
83	Linear	0.9863	4.38	-5.20	0.001
	Logarithmic	0.8966	44.23	-6.99	0.01
	Power	0.9965	1.30	0.27	0.001

Table 3:7. Statistical parameters of the regression analysis of the stimulus response relationship for the units which did not display a peak response within the range of heat stimuli tested. Symbols used in the table are the same as those used in table 3:5.

Unit	Intensity Function	r	b	A	P(<)
40	Linear	0.6594	1.59	-1.68	NS
	Logarithmic	0.4044	9.48	1.49	NS
	Power	0.1904	0.19	0.69	NS
41	Linear	0.9769	12.96	-14.24	0.001
	Logarithmic	0.9093	117.60	-15.23	0.01
	Power	0.9554	1.40	0.65	0.001
46	Linear	0.9856	27.25	-33.25	0.01
	Logarithmic	0.9153	176.95	-13.66	0.05
	Power	0.9973	1.95	0.60	0.001
48	Linear	0.8747	2.86	-8.28	0.01
	Logarithmic	0.6874	28.08	-7.54	0.05
	Power	0.8851	1.06	0.11	0.01
49	Linear	0.9440	5.67	12.81	0.01
	Logarithmic	0.9905	58.03	7.99	0.001
	Power	0.9639	0.88	1.00	0.001
54	Linear	0.9443	6.14	1.29	0.001
	Logarithmic	0.9417	68.12	-5.64	0.001
	Power	0.9724	1.23	0.58	0.001
55	Linear	0.2379	0.21	3.46	NS
	Logarithmic	0.0458	0.50	5.5	NS
	Power	0.1933	0.18	0.79	NS
56	Linear	0.9961	9.07	1.93	0.001
	Logarithmic	0.9038	102.78	-6.53	0.001
	Power	0.9805	0.81	1.14	0.001
58	Linear	0.9255	6.79	-17.55	0.01
	Logarithmic	0.7631	54.56	-13.36	NS
	Power	0.9470	1.81	-0.25	0.01
60	Linear	0.8637	4.71	-14.86	0.01
	Logarithmic	0.6862	41.65	-12.39	NS
	Power	0.9154	1.66	-0.35	0.01

61	Linear	0.9175	3.20	-8.09	0.01
	Logarithmic	0.7616	29.51	-7.34	0.05
	Power	0.9577	1.44	-0.17	0.001
62	Linear	0.9039	6.66	-18.28	0.01
	Logarithmic	0.7291	52.36	-13.39	NS
	Power	0.7185	1.40	0.02	NS
67	Linear	0.9445	3.90	3.90	0.02
	Logarithmic	0.9914	28.62	5.04	0.001
	Power	0.9798	0.93	0.74	0.01
72	Linear	0.8885	3.55	-8.59	0.01
	Logarithmic	0.7239	32.20	-7.31	0.05
	Power	0.9153	1.15	0.15	0.01
73	Linear	0.9285	7.95	-17.34	0.001
	Logarithmic	0.7994	76.08	-17.48	0.02
	Power	0.9953	1.75	5.56	0.001
76	Linear	0.9762	4.64	-5.79	0.001
	Logarithmic	0.9045	47.84	-8.35	0.01
	Power	0.9737	1.66	-0.05	0.001
79	Linear	0.8777	4.99	-13.75	0.01
	Logarithmic	0.7021	38.89	-9.87	NS
	Power	0.9113	1.59	-0.24	0.01

Table 3:8. Population correlation coefficient and coefficient of determination for each of the three intensity functions which were fitted to the data. For each function the relevant values of r and r^2 were pooled and the arithmetic mean calculated. The figures in the body of the table represent the mean $\pm$SE. The values are given for both the 25 units which displayed a peak response and for the 17 units which did not display a peak response, and for all 42 units.

POPULATION CORRELATION COEFFICIENT, r

Intensity Function	Units Tested to Peak Response	Units Not Tested to Peak Response	All Units
n	25	17	42
Linear	0.9141±0.0252	0.8732±0.0438	0.8976±0.0231
Logarithmic	0.8301±0.0309	0.7564±0.0569	0.8003±0.0296
Power	0.8833±0.0335	0.8501±0.0621	0.8699±0.0317

POPULATION COEFFICIENT OF DETERMINATION, r^2

n	25	17	42
Linear	0.8510±0.0379	0.7933±0.0555	0.8276±0.0317
Logarithmic	0.7120±0.0471	0.6241±0.0656	0.6764±0.0387
Power	0.8072±0.0484	0.7845±0.0734	0.7980±0.0409

IV. DISCUSSION

The results presented in this chapter demonstrate the presence of cutaneous nociceptors in the amputated beak stump of beak-trimmed chickens. These nociceptors are, in their general characteristics, very similar to those described in the intact beak in Chapter 2 of this thesis. They are of two types, heat-responsive and heat and mechanically-responsive. They have the ability to encode in their discharge information relating to heat intensity. The heat thresholds are not significantly different from those in the intact beak.

Examination of other parameters of the heat stimulus-response relationship reveals significant differences between the two populations of nociceptors. Thus the temperatures at which the peak/maximal responses were obtained, and the temperature range were higher, and the peak/maximal responses and sensitivities were lower, for the nociceptors recorded from the amputated beak stumps. The significance of these differences between the two nociceptor populations must be viewed with some caution. Within both populations of nociceptors there were two groups of units, those tested to peak response and those not tested to peak response. Pooling the data from both of these groups involves the assumption that the maximal responses for the units not tested to peak approximate to the values of the true peak response. The possible biasing effects of this assumption

are unknown. It is pertinent to note that comparing only the units tested to peak response for both populations, no significant differences were found for any of the heat stimulus response parameters.

An interesting contrast with these results is provided by the work of Dickhaus et al (1976a,b), the only other comparative study of nociceptors in normal and pathological situations. They examined the heat stimulus-response characteristics of regenerating C-fibre innervated nociceptors in the cat foot pad after nerve crush. Compared to the nociceptors in the normal preparation (Beck et al, 1974), the population of regenerating nociceptors showed a decrease in the mean heat threshold and an increase in the mean sensitivity. None of these units were tested to peak response so their estimate of sensitivity contains the same type of assumption used in the present study. Bearing in mind these limitations, it is interesting that the present results do not agree with those of Dickhaus et al (1976a,b). One possible source of the discrepancy is sample size. Given the large variation in sensitivities between individual nociceptors in both the data of Dickhaus et al (1976a,b) and the present data, it is desirable to obtain a large sample to encompass this variation. A small sample could conceivably contain a bias towards the upper or lower end of the sensitivity range. Dickhaus et al (1976a,b) used a smaller sample (12 units from the normal and regenerated nerves) than

the present investigation, so this could be a factor underlying the discrepancy (Zimmermann, personal communication 1984).

It is, perhaps, misleading to compare the present results with those of Dickhaus et al (1976a,b) because the preparations involved are very different. The area investigated by them was denervated, then studied at various times after nerve regeneration. The present preparation consisted of an amputation stump. Anatomical studies (Breward and Gentle, 1985) of trimmed beaks indicate that the cauterization causes immediate damage to the beak tissue including the alveolar mandibular nerve for up to 3mm proximal to the tip of the amputation stump. By six days after trimming this damaged portion had degenerated, by ten days there was evidence of nerve regrowth and enlargement of the terminal portion of the nerve, and at 15-30 days there was a neuroma present at the end of the nerve stump together with numerous bundles of regenerating fibres. These fibres continued to grow but, because of the adjacent scar tissue, were unable to innervate dermal structures. The fibres grew back on themselves to form a complex mass of intertwining regenerating fibres together with the surrounding tissue.

The relationship of post-injury neuroma formation to abnormal afferent discharge is discussed in Chapter 4 (Discussion). Given the complexity of the processes likely to occur in the

tissues of the beak stump after beak trimming it is likely that the nociceptive discharges described here had their origin in a variety of structures. Possible candidates include both intact receptors located in parts of the beak undamaged by beak trimming, regenerating fibres which were sensitive to heat, or lastly, fibres which had established connections with a target structure to form functionally regenerated nociceptors.

From this, one would expect to find differences in the properties of nociceptors related to two variables: (a) the receptive field location in relation to the distal tip of the amputated beak stump. and (b) the time after amputation at which the units were recorded. This would require repeated sampling of nociceptors at specified receptive field locations at different times after beak trimming, and vice versa. This would be technically difficult, due to the serendipitous nature of the process of single-unit dissection of nociceptor afferents. The present data consist of units from different locations at different times after beak trimming, which prohibited location/time analysis. It may well be that this type of analysis would reveal differences in individual stimulus-response characteristics that are not obvious from the present data.

An interesting phenomenon was the bursting discharge pattern observed during heat stimulation. This was noted for

nociceptors in the trimmed beak more frequently than for normal nociceptors (38.3% and 5.6% respectively). The significance of this increase in bursting activity is unknown. Bursting discharges in response to heat stimulation have been noted for normal nociceptors by other authors, eg. in the cat (Bessou and Perl, 1969; Belmonte and Giraldez, 1982) and primate (Croze et al, 1976; Kumazawa and Perl, 1977). The incidence of bursting type nociceptors in the trimmed beak could not be correlated with the time after trimming at which they were recorded, or the location of the receptive field. It is possible that the greater incidence of this type of discharge reflects a sampling bias.

Chapter 4.

SPONTANEOUS AFFERENT ACTIVITY RECORDED FROM THE TRIMMED BEAK OF THE CHICKEN

4. SPONTANEOUS AFFERENT ACTIVITY RECORDED FROM THE TRIMMED BEAK OF THE CHICKEN

I. INTRODUCTION

See Introduction to Chapter 3.

II. METHODS

Methods were as described in Chapter 3.

III. RESULTS

A resting discharge was present in 96 units, recorded from the alveolar mandibular nerve of beak-trimmed chickens, in the absence of any applied stimulus. This is referred to as spontaneous activity. Units showing spontaneous activity were recorded from chickens prepared for electrophysiological experiments from 1-83 days after beak trimming. Table 4:1 lists all 96 units obtained in the order in which they were recorded, along with their receptive field locations, discharge patterns, and responses to stimulation of the beak.

Receptive fields

Receptive fields were located for 60 of the 96 spontaneously active units. Of the remaining 36 units, the receptive fields of 32 could not be located using mechanical, heat and cold search stimuli, and 4 units were not tested. The distribution

of the receptive fields located is illustrated in Table 4:2. The majority (52) of the receptive fields were located 0-6 mm from the tip of the beak stump, 7 of them on the tip itself. The receptive fields of the other 8 units were located 7-14 mm from the tip.

The pattern of the spontaneous discharge

The spontaneous discharge was recorded for a minimum of 20 minutes, maximum 90 minutes. Three types of spontaneous discharge pattern were observed: "regular","irregular" and "bursting." The discharge pattern of each unit is given in Table 4:1. For each of the 96 units, the pattern of the spontaneous discharge was similar throughout the recording period.

(i) Regular

Twelve of the 96 units (12.5%) carried a continuous regular spontaneous discharge, a typical example of which is illustrated in fig 4:1. The maximum instantaneous frequency of the discharge varied between units, ranging from 1 to 16 impulses/sec..

(ii) Irregular

Seventy-five of the 96 units (78.1%) exhibited a continuous and irregular spontaneous discharge, examples of which are illustrated in figs 4:1 and 4:2. No patterns could be detected by visual inspection. The maximum instantaneous frequency of the discharge varied from unit to unit ranging from <1 to 23 impulses/sec.

(iii) Bursting

Nine of the 96 units (9.4%) showed a bursting spontaneous discharge pattern, (see figs 4:1 and 4:2). This pattern of discharge consisted of intermittent bursts of activity with variable periods of silence between bursts. The bursts were of variable duration and within the burst the discharge was usually regular.

For each of the 96 units, the pattern of the spontaneous discharge was similar throughout the recording period.

The effects of cutaneous stimulation of the beak on the spontaneous discharge.

Ninety two of the 96 spontaneously discharging units were tested for their response to cutaneous stimulation. Seventy eight of these 92 units were tested with mechanical, cold and

heat stimulation. The remaining 14 units were tested with mechanical and cold stimulation only.

The responses to the different stimulus modalities varied from unit to unit (see table 4:1). Where all 3 stimulus modalities were used the responses were classified into 13 categories. These categories are given in table 4:3, along with the spontaneous discharge pattern, the receptive field location, and the time after beak trimming at which the units were recorded. The categories ranged from one in which all 3 stimulus modalities exerted an excitatory effect on the discharge frequency (3 units) through to one in which 2 modalities (mechanical and heat) had an inhibitory effect (2 units). The largest category was that in which none of the stimuli, applied anywhere on the beak surface, had any effect on the ongoing discharge (32 units). The next largest category was that in which cold had an excitory effect, heat had an inhibitory effect and mechanical stimulation was ineffective (13 units). In this latter category, 6 units carried a regular spontaneous discharge and 7 an irregular spontaneous discharge.

Examples of the effects of the 3 stimulus modalities on the different types of ongoing discharge are illustrated in figs 4:3 to 4:8.

37 units were responsive to mechanical stimulation of the receptive field. The effect of mechanical stimulation was excitatory for 35 units, the spontaneous discharge of 2 units being inhibited by this mode of stimulus. For the mechanically excitable units, a sustained mechanical displacement of the receptive field evoked an increased discharge frequency for the duration of the stimulus. The pattern of this discharge varied between units, examples of which are shown in figs.4:5 and 4:6. Upon removal of the stimulus, the discharge frequency returned to its pre-stimulus level. Mechanical stimulation inhibited the discharge of 2 units, one of which is illustrated in fig.4:7. This inhibition was reversible upon removal of the stimulus in both cases.

40 units were responsive to cold stimulation of the receptive field. The effect was excitatory for all these units. In general, cooling the receptive field produced an initial increase in discharge frequency, the frequency declining during the period over which the temperature was maintained at the lower level. Representative examples are shown in figs.4:3, 4:4 and 4:7. A change of the pattern of the discharge during cold stimulation, as shown in figs. 4:4 and 4:7, was typical for most of the units. Passive rewarming of the receptive field back to the pre-stimulus temperature produced a cessation of the discharge, which gradually increased in frequency back to the original pre-stimulus level

and pattern. More complex after-effects of cooling were noted for the units which had a bursting spontaneous discharge (fig.4:7). After rewarming the receptive field back to the original temperature, the discharge pattern of these units alternated between bursting and regular for up to 5 minutes after the termination of the stimulus, the discharge then resuming its bursting pattern.

41 units were responsive to heating the receptive field. The effect was inhibitory for 22 units and excitatory for 19 units. For the heat-inhibited units heating to 36°C or above abolished the discharge. This effect was reversible upon recooling back to the pre-stimulus temperature (figs.4:4 and 4:7). Changes in the discharge pattern after heat stimulation were noted for the bursting units. This change took the form of a post-stimulus alternation between bursting and regular discharge, similar to that observed after cold stimulation.

An increase in discharge frequency in response to heating the receptive field above 34°C was observed for 19 units (figs.4:5, 4:6 and 4:8). The effect was reversible in all cases. However, long lasting (up to 3 minutes) after-discharges at a frequency higher than the pre-stimulus level were noted for 10 of the units (fig.4:5).

17 spontaneously discharging units which were excited by heat

were tested with a series of different temperature levels in a similar manner to that described for the nociceptors (C, $A\delta$) (Chapters 2 and 3). All 17 units exhibited a heat dependence, increasing the temperature producing an increased response. A representative example is shown in fig.4:8. Both units illustrated had an irregular spontaneous discharge, one discharging single impulses, the other single impulses or short bursts of 2 - 3 impulses. Heating the receptive field produced an increase in discharge frequency in both units, the increase being more dramatic for the "bursting" unit. Neither unit exhibited any change in discharge pattern during heat stimulation. Both units displayed an increased frequency of discharge with increase of stimulus temperature.

Fig.4:1. Three examples of spontaneous afferent activity recorded from the amputated beak stump of beak-trimmed chickens.

(a) Unit with a regular discharge pattern, recorded 36 days after amputation. The receptive field was located 1mm. proximal to the tip of the amputation stump. Unit no.58

(b) Unit with an irregular discharge pattern, recorded 37 days after amputation. The receptive field could not be located using heat, cold or mechanical stimulation. Unit no.73

(c) Unit with a bursting discharge pattern, recorded 31 days after amputation. Note the irregularity of occurrence and duration of the bursts, and the regularity of the discharge pattern within the bursts. The receptive field was located 3mm. proximal to the tip of the amputation stump. Unit no.56

Calibration bar (time)= 1 sec. in a and c, 5 sec in b.

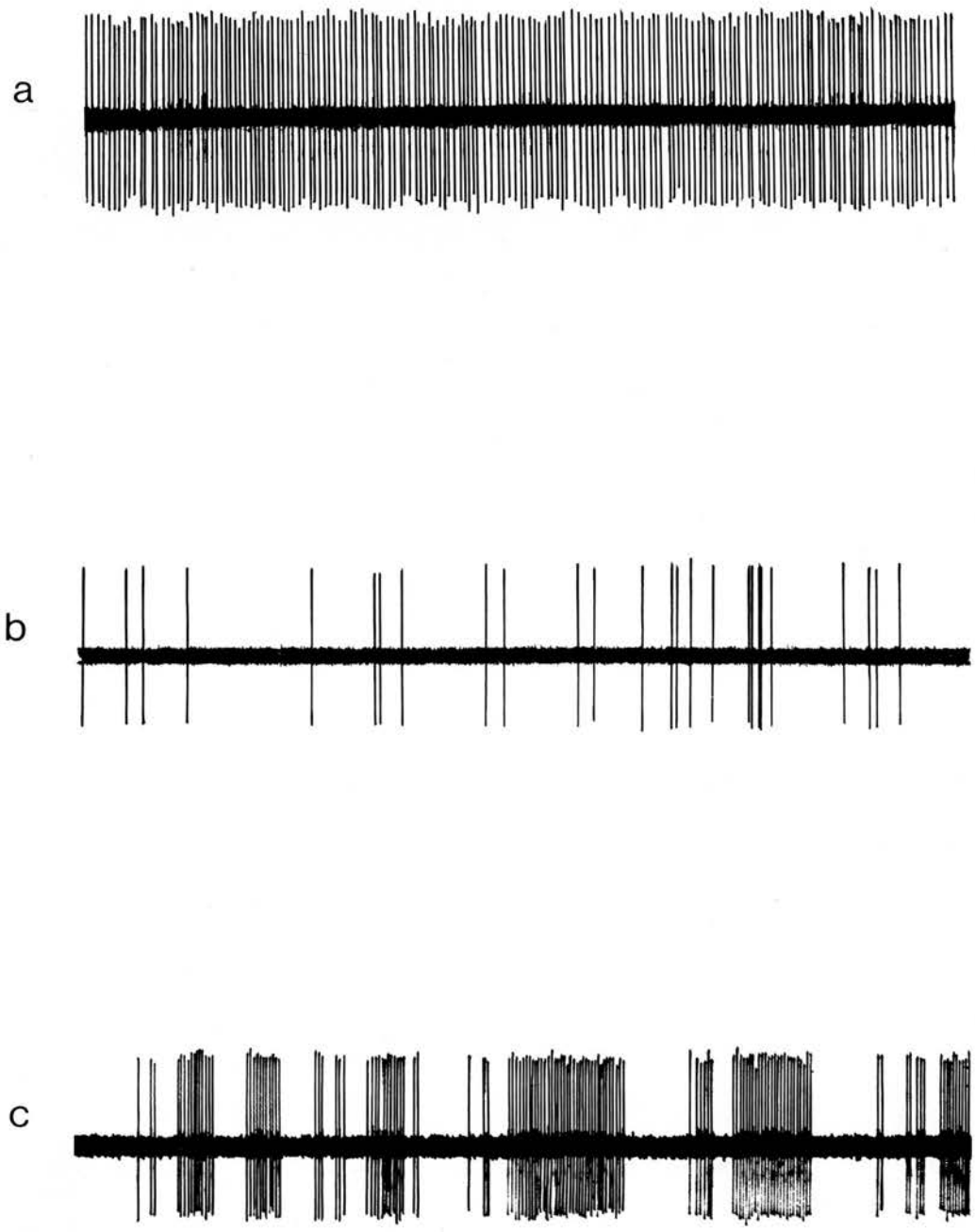

a
b
c

Fig. 4:2. Three examples of spontaneous afferent activity recorded from the amputated beak stump of beak-trimmed chickens.

In each of these three examples, and in all subsequent illustrations, the upper trace is the afferent impulse activity and the lower trace is a dot display representing the instantaneous firing frequency (the reciprocal of the interspike interval) of the unit.

(a) Unit with an irregular discharge pattern, recorded 37 days after amputation. The receptive field could not be located using heat, cold, or mechanical stimulation. Unit no.75

(b) Unit with an irregular discharge pattern, recorded 36 days after amputation. The receptive field was located on the tip of the amputation stump. Unit no.59

(c) Unit with a bursting discharge pattern, recorded 31 days after amputation. The receptive field could not be located using heat, cold or mechanical stimulation. Unit no.57

Horizontal calibration bar (time)= 2 sec. in a, 1 sec. in b and c.

Vertical calibration bar (discharge frequency) = 5 ips. in a and b and 10 ips in c.

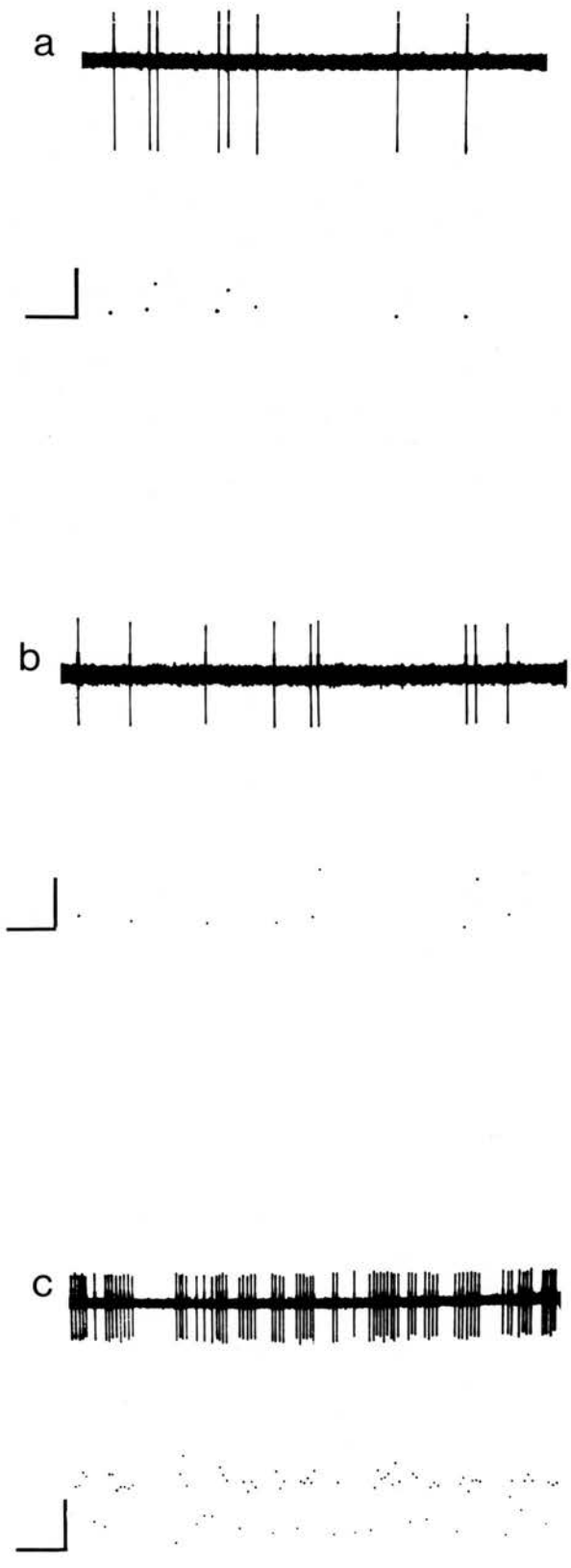

a
b
c

Fig. 4:3. The effect of cold stimulation on the ongoing activity of a regularly discharging unit.

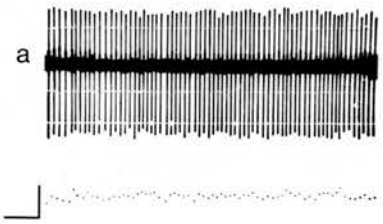
(a) Record of unprovoked spontaneous activity.

(b) The effect of ccoling the receptive field of the unit from 29°C to 19°C. The discharge frequency increased from 10 ips to 44 ips during the temperature change. The temperature was maintained at 19 °C for 23 sec.. The discharge frequency declined within this time to a lower level (maximum 8 ips) and its pattern was less regular. Rewarming the receptive field from 19°C to 29 °C caused a decrease in the discharge frequency followed by a cessation of the discharge. The discharge reappeared after approximately 10 sec. and its frequency recovered back to the original, prestimulus, level.

This unit was recorded 22 days after amputation. Its receptive field was located 1mm. proximal to the tip of the amputation stump. Unit no. 43

Horizontal calibration bar (time) = 1 sec. in a, 5 sec. in b. Vertical calibration bar (discharge frequency) = 10 ips in a, 20 ips in b.

Note the reduced gain on the impulse activity record in b.


a

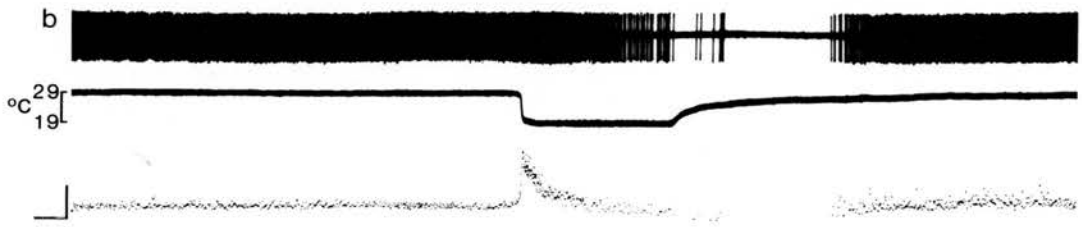

b
°C 29
19

Fig. 4:4. The effect of cold and heat stimulation on the ongoing activity of a regularly discharging unit.

(a) Record of unprovoked spontaneous activity.

(b) Effect of cooling the receptive field of the unit from 29 °C to 24°C The discharge frequency increased and the pattern of the discharge changed from regular to bursting (maximum firing frequency 44 ips). Rewarming the receptive field from 24°C to 29 °C caused a decrease in discharge frequency followed by a cessation of the discharge. After 10 sec. the discharge reappeared and its frequency recovered to the original, prestimulus, level in 25 sec..

(c) Effect of heating the receptive field of the unit from 29 °C to 32°C then from 32 °C to 35°C Heating from 29°C to 32°C caused an initial cessation of the discharge. After 5 sec., the discharge reappeared, its pattern changed to a bursting pattern (maximum firing frequency 10 ips). After 50 sec. the discharge returned to a more regular pattern. Warming the receptive field from 32°C to 35 °C caused cessation of the discharge. On recooling the receptive field back to 32°C the regular (10ips) discharge reappeared. Cooling back to the original temperature (29 °C) caused an initial increase in discharge frequency (maximum 18 ips), which declined to the original ongoing frequency of 10 ips in 60 sec..

This unit was recorded 30 days after amputation. Its receptive field was located 2mm from tip of the amputation stump. Unit no. 54.

Horizontal calibration bar (time) = 1 sec. in a, 5 sec. in b and c. Vertical calibration bar (discharge frequency)=5 ips in a, 10 ips in b and c.

Note the reduced gain on the impulse activity record in traces b and c.

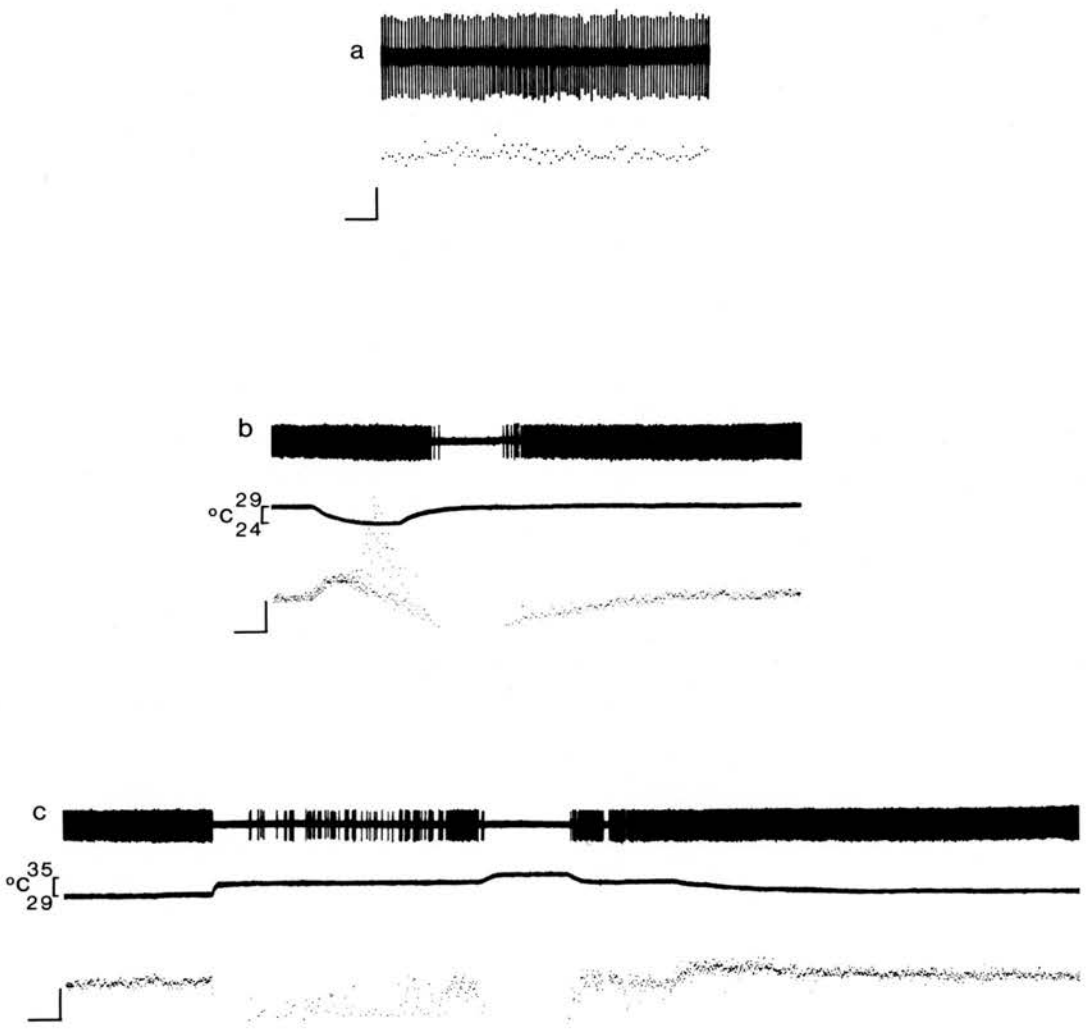

a
b
°C 29
24
c
°C 35
29

Fig. 4:5. The effect of mechanical and heat stimulation on the spontaneous activity of an irregularly discharging unit.

(a) The first part of this trace illustrates the unprovoked spontaneous activity. The discharge was slow (<2 ips) with one short burst present. Mechanical displacement of the receptive field was effected with a 50 g. von Frey hair, the stimulus duration indicated by the horizontal bars under the record. The first mechanical stimulus, maintained for 10 sec., provoked a high frequency (maximum 30 ips) discharge which was sustained for the duration of the stimulus. This discharge ceased upon removal of the stimulus, and the original, prestimulus, discharge reappeared. Repetition of the stimulus provoked a similar response, followed by recovery.

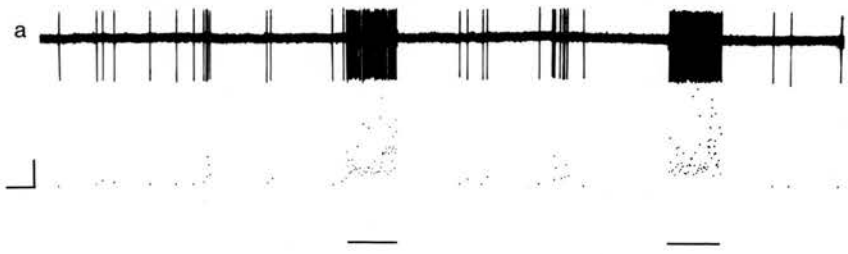
(b) The temperature of the receptive field was raised from 29 °C to 34 °C with no observable effect on the spontaneous discharge. This record illustrates the effect of raising the temperature from 34°C to 45°C. A high frequency (maximum 50 ips) bursting discharge was provoked. Recooling the receptive field back to 34°C caused a decrease in the discharge frequency (maximum 26 ips). The bursting activity ceased within 25 sec. of the termination of the stimulus, but a low frequency, regular, discharge persisted until approximately 130 sec. after the termiation of the stimulus, its frequency declining during this time. The original, prestimulus, irregular discharge then reappeared.

This unit was recorded 30 days after amputation. Its receptive field was located 6mm. proximal to the tip of the amputation stump. Unit no.52.

Horizontal calibration bar (time) = 5 sec.

Vertical calibration bar (discharge frequency)=10 ips.

Note the reduced gain on the impulse activity record in trace b.


a

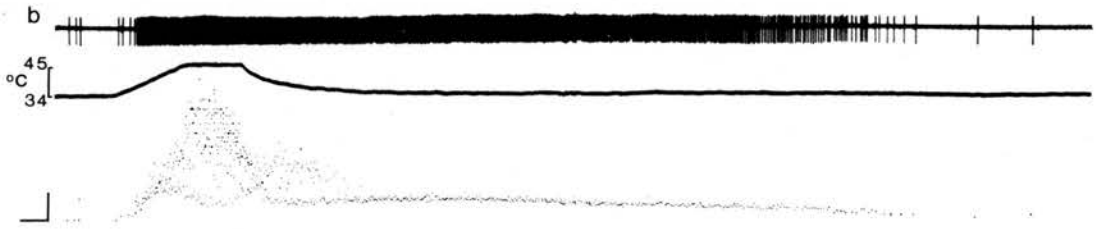

b
45
°C
34

Fig. 4:6. The effect of mechanical and heat stimulation on the spontaneous activity of an irregularly discharging unit.

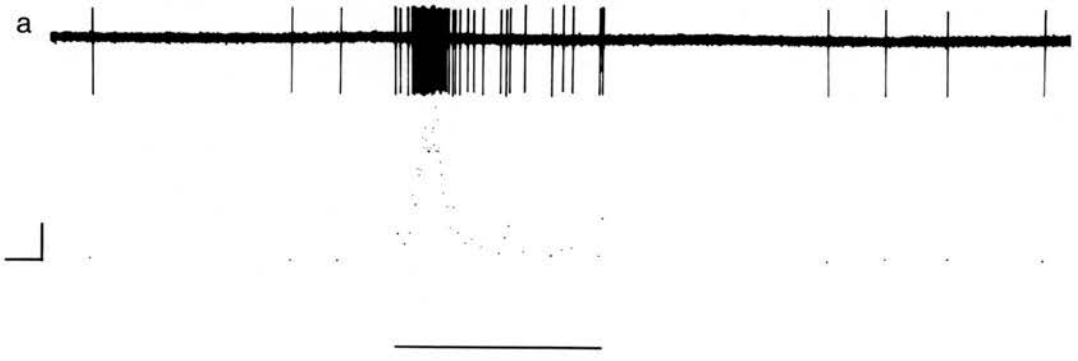
(a) The first part of this trace illustrates the unprovoked spontaneous activity. The discharge was slow (<1 ips) and irregular. A sustained mechanical displacement of the receptive field with a 50 g. von Frey hair (indicated by the bar under the record) provoked an increase in the discharge frequency. This discharge reached a peak (maximum frequency 45 ips) then declined to a low frequency irregular discharge which was maintained for the duration of the stimulus. The original, prestimulus, discharge appeared after the removal of the stimulus.

(b) The temperature of the receptive field was raised from 29 °C to 34 °C with no observable effect on the spontaneous discharge. This record illustrates the effect of raising the temperature from 34 °C to 52 °C. An irregular bursting discharge (maximum frequency 10 ips) was provoked. This discharge was maintained for the duration of the stimulus. The discharge ceased on recooling the receptive field back to 34 °C and the original, prestimulus, discharge reappeared.

This unit was recorded 38 days after amputation. Its receptive field was located 12 mm. proximal to the tip of the amputation stump. Unit no.77.

Horizontal calibration bar (time) = 1 sec. in a, 5 sec. in b.

Vertical calibration bar (discharge frequency) = 10 ips. in a, 5 ips in b.


a

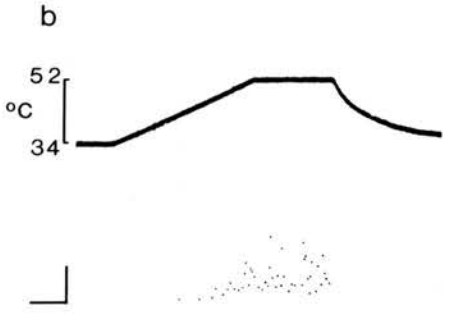

b
52
°C
34

Fig. 4:7. The effect of mechanical, cold and heat stimulation on the spontaneous activity of a unit with a bursting discharge.

(a) Unprovoked spontaneous activity. The bursts were irregular in occurrence and duration and the discharge frequency was regular within the bursts.

(b) A sustained mechanical displacement of the receptive field with a 50g von Frey hair (indicated by the bar under the record) caused a cessation of the discharge for the duration of the stimulus. The discharge resumed immediately upon removal of the stimulus.

(c) Cooling the receptive field of the unit from 32°C to 28°C provoked a regular discharge which was maintained for the duration of the stimulus. The frequency of this discharge was initially higher (maximum frequency 22 ips.), during the temperature change, than the prestimulus discharge frequency. The frequency then declined to a regular (maximum frequency 12 ips) discharge for the duration of the 28 °C temperature stimulus. On rewarming the receptive field back to 32°C the discharge slowed down, and stopped for 2 sec.. The original, prestimulus, bursting discharge then reappeared, the bursts being longer in duration than prestimulus values. The unit then discharged in an uninterrupted regular pattern for several minutes after the end of this record.

(d) Several minutes after the cold stimulation illustrated in (c), the unit was still discharging in a regular pattern. Raising the temperature of the receptive field from 32°C to 34 °C provoked an immediate reduction in discharge frequency to a low frequency (<4 ips.) irregular discharge with occasional short bursts superimposed. Raising the temperature of the receptive field from 34 °C to 36°C produced a cessation of the discharge. Recooling the receptive field from 36 °C to 34°C resulted in the reappearance of the low frequency (<4 ips) irregular discharge with occasional short bursts evident. Recooling the receptive field from 34°C to 32°C resulted in the reappearance of the original, prestimulus, bursting discharge followed by a regular discharge which persisted for several minutes after the end of this record. The unit then resumed its bursting discharge.

This unit was recorded 31 days after amputation. Its receptive field was located 3mm proximal to the tip of the amputation stump. Unit no.56.

Horizontal calibration bar (time) = 1 sec in a, 2 sec in b, 5 sec in c and d.

Vertical calibration bar (discharge frequency) = 10 ips.

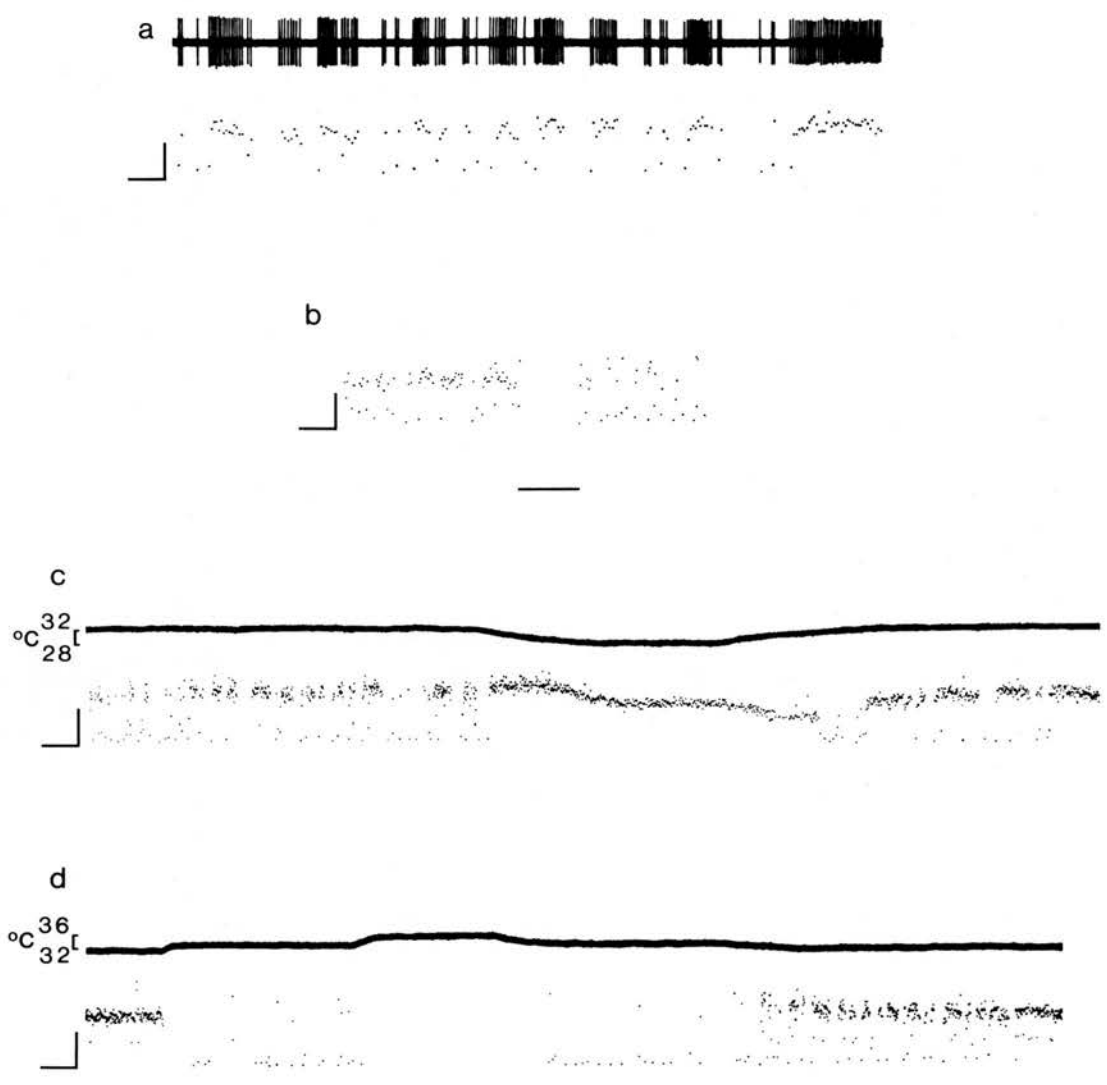

a
b
c
°C 32
28
d
°C 36
32

Fig. 4:8. The effect of heat stimulation on the spontaneous activity of two units recorded simultaneously from the same nerve filament.

(a) Unprovoked spontaneous activity. Two units are present. One (the larger unit) discharged irregularly, the other discharged in short bursts at irregular intervals.

(b) to (i). These traces illustrate the effect of heating the receptive field to different temperatures on the spontaneous discharge of the two units. The temperature of the receptive field was first raised to 34°C, then heat stimuli at different temperatures were delivered in random sequence, with 3 minutes between successive stimuli. The temperature of each stimulus is indicated on the left of the trace.

Note the increased discharge from both units with increasing temperature. The magnitude of the response is much greater for the smaller unit. The prestimulus discharge pattern was resumed by both units after each stimulus.

These two units were recorded 22 days after amputation. Their receptive field was located 7mm proximal to the tip of the amputation stump. Unit nos.44,45.

Horizontal calibration bar (time) = 5 sec in a, 3.3 sec in b to i inclusive.

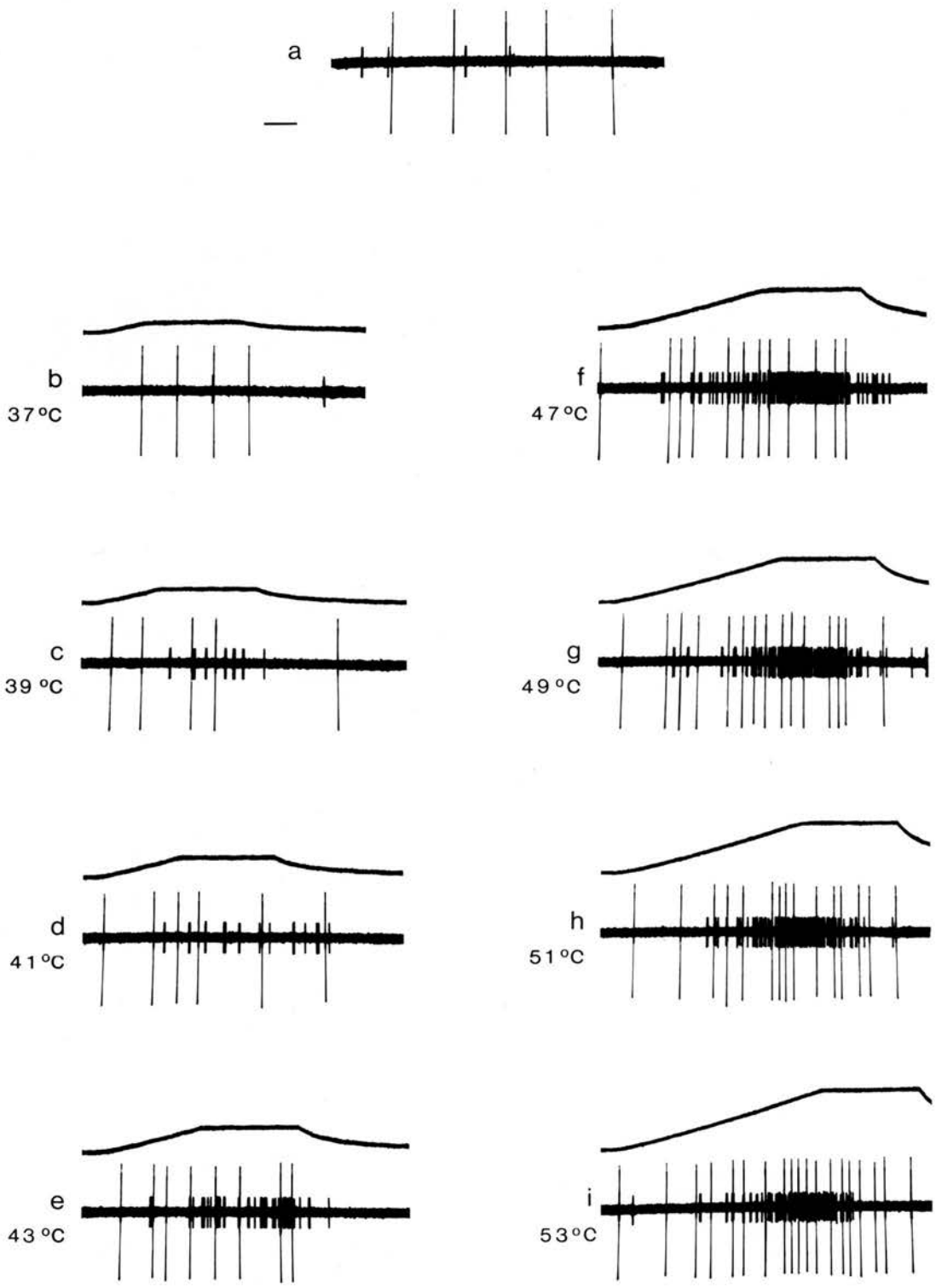

a
b
37°C
c
39 °C
d
41°C
e
43 °C
f
47°C
g
49 °C
h
51°C
i
53°C

Table 4:1. Spontaneously discharging units recorded from the amputation stump of beak-trimmed chickens. 96 units were recorded. They are listed in order of the time after beak-trimming at which they were recorded.

Receptive field (RF) location refers to the distance (measured to the nearest 0.5mm.) from the distal tip of the beak stump to the distal edge of the receptive field. In this connection :

0 indicates that the receptive field was located on the tip of the stump;
NL= receptive field could not be located using heat, cold, or mechanical stimulation.
R = regular discharge pattern.
I = irregular discharge pattern
B = bursting discharge pattern
+ = stimulus had an excitory effect on the spontaneous discharge, i.e. it provoked an increase in the discharge frequency.
- = stimulus had an inhibitory effect on the spontaneous discharge, i.e. it provoked a decrease in the discharge frequency.
NE= stimulus had no effect on the spontaneous discharge.
NT= stimulus was not tested.

Unit number	Time after operation (days)	RF location (mm from tip)	Spontaneous firing pattern	RESPONSE TO STIMULATION		
				Heat	Cold	Mechanical
1	1	3	I	NT	NE	+
2	5	2	B	+	+	+
3	5	4	B	NE	+	NE
4	5	4	I	NE	+	NE
5	6	0	I	-	+	NE
6	6	0	I	-	+	NE
7	6	0	I	-	+	NE
8	6	0	I	-	+	NE
9	6	NL	I	NE	NE	NE
10	7	11	I	+	NE	+
11	7	4	R	-	+	NE
12	12	2	I	-	+	-
13	12	2	R	-	+	+
14	13	NL	I	NE	NE	NE
15	13	NL	I	NE	NE	NE
16	13	NL	I	NE	NE	NE
17	14	2	R	NT	+	+
18	14	NL	I	NE	NE	NE
19	14	NL	I	NE	NE	NE
20	14	14	I	NT	+	+
21	14	14	I	NT	+	+
22	14	14	I	NT	+	+
23	15	6	I	+	NE	+
24	15	NL	R	NE	NE	NE
25	15	NL	R	NE	NE	NE
26	15	NL	I	NE	NE	NE
27	15	3	I	+	NE	+
28	15	NL	I	NE	NE	NE
29	15	4	I	NE	NE	+
30	18	NL	I	NE	NE	NE
31	18	NT	B	NT	NT	NT
32	18	NT	I	NT	NT	NT
33	18	NT	B	NT	NT	NT
34	20	5	I	+	NE	+
35	20	5	I	+	NE	NE
36	20	5	I	+	NE	+
37	21	3	I	+	NE	+
38	21	NT	R	NT	NT	NT
39	21	2	I	-	NE	+
40	21	2	R	-	+	NE

Unit number	Time after operation (days)	RF location (mm from tip)	Spontaneous firing pattern	RESPONSE TO STIMULATION		
				Heat	Cold	Mechanical
41	21	2	I	+	+	NE
42	22	1	I	+	NE	+
43	22	1	R	-	+	NE
44	22	7	I	+	NE	+
45	22	7	B	+	NE	NE
46	22	6	B	NE	+	+
47	22	6	I	-	NE	NE
48	24	3	I	NT	+	NE
49	24	3	I	NT	+	NE
50	30	0	I	+	NE	+
51	30	10	I	+	+	+
52	30	6	I	+	NE	+
53	30	6	I	+	NE	+
54	30	2	R	-	+	NE
55	31	0	I	NE	+	NE
56	31	3	B	-	+	-
57	31	NL	B	NE	NE	NE
58	36	1	R	-	+	NE
59	36	0	I	-	+	+
60	37	2	I	-	+	NE
61	37	2	I	-	+	NE
62	37	NL	I	NE	NE	NE
63	37	NL	I	NE	NE	NE
64	37	NL	I	NE	NE	NE
65	37	NL	I	NE	NE	NE
66	37	NL	I	NE	NE	NE
67	37	NL	I	NE	NE	NE
68	37	NL	I	NE	NE	NE
69	37	NL	I	NE	NE	NE
70	37	6	I	NT	+	+
71	37	6	I	NT	+	+
72	37	NL	I	NE	NE	NE
73	37	NL	I	NE	NE	NE
74	37	NL	I	NE	NE	NE
75	37	NL	I	NE	NE	NE
76	38	1	I	-	+	NE
77	38	12	I	+	+	+
78	38	5	I	NT	+	+
79	38	5				

Unit number	Time after operation (days)	RF location (mm from tip)	Spontaneous firing pattern	RESPONSE TO STIMULATION		
				Heat	Cold	Mechanical
80	38	5	I	NT	+	+
81	38	4	I	NT	NE	+
82	38	4	I	NT	+	+
83	38	NL	I	NE	NE	NE
84	39	4	I	-	+	+
85	39	4	B	-	+	+
86	39	NL	I	NE	NE	NE
87	39	NL	I	NE	NE	NE
88	53	2	I	+	NE	+
89	53	2	I	+	NE	NE
90	62	NL	I	NE	NE	NE
91	62	NL	I	NE	NE	NE
92	64	NL	I	NE	NE	NE
93	71	NL	R	NE	NE	NE
94	83	4	I	-	+	+
95	83	4	R	-	+	NE
96	83	NL	I	NE	NE	NE

Table 4:2 The distribution of the receptive fields of the sponstaneously active units recorded from beak-trimmed chickens. Receptive field location refers to the distance from the distal tip of the beak stump to the distal edge of the receptive field. 0 indicates that the receptive field was located on the tip of the stump. Each cross represents one unit.

Note that receptive fields were located for 60 out of the sample of 96 spontaneously active units. Of the remaining 36 units of the sample, the receptive fields of 32 could not be located with either mechanical, cold, or heat search stimuli, and 4 units were not tested.

RECEPTIVE FIELD LOCATIONS
(mm. proximal to tip of beak stump)

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
X	X	X	X	X	X	X	X			X	X	X		X
X	X	X	X	X	X	X	X							X
X	X	X	X	X	X	X								X
X	X	X	X	X	X	X								
X		X	X	X	X	X								
X		X	X	X	X	X								
X		X		X		X								
		X		X										
		X		X										
		X		X										
		X												
		X												

Table 4:3. This table lists seventy-eight spontaneously discharging units which were tested for responsiveness to all three stimulus modalities (heat,cold, and mechanical). The units are divided into thirteen categories on the basis of their responsiveness to the different stimulus modalities. Symbols and abbreviations used in this table are identical to those of table 4:1.

Number of units	RESPONSE TO STIMULATION			Spontaneous firing pattern	Time after operation (days)	RF location (mm from ti
	Heat	Cold	Mechanical			
3	+	+	+	I,B	5-38	2-12
1	+	+	NE	I	21	2
12	+	NE	+	I	7-53	0-11
1	NE	+	+	B	22	6
3	+	NE	NE	I,B	20-53	2-7
3	NE	+	NE	I,B	5-31	0-4
1	NE	NE	+	I	15	4
32	NE	NE	NE	R,I,B	6-83	NL
1	-	NE	NE	I	22	6
1	-	NE	+	I	21	2
13	-	+	NE	R,I	6,83	0-4
5	-	+	+	R,I,B	12-83	0-4
2	-	+	-	I,B	12-31	2-3

IV. DISCUSSION

The results presented in this chapter demonstrate that from 1 to 83 days after beak trimming, spontaneous afferent activity can be recorded from the alveolar mandibular nerve of the chicken beak. This activity can be contrasted with the spontaneous activity found in the intact beak. The spontaneous activity observed in the intact beak consisted of units which were classified as cold receptors. These cold receptors had easily identifiable characteristics i.e. a regular spontaneous discharge, which was excited by cold, inhibited by heat and unaffected by mechanical stimulation. In the sample of 96 spontaneously active units recorded from the beak stump, 78 units were tested with all three stimulus modalities. Thirteen of these units responded in a similar way to normal cold receptors. However, the remainder of the units did not show characteristics similar to those of any of the normal receptors.

The origin of the spontaneous activity recorded from the trimmed beak

Extensive work on mammals has demonstrated that peripheral nerve injury induces several processes which lead to the production of abnormal spontaneous afferent activity. One of these processes, neuroma formation, has been the focus of attention in recent years. A review of current knowledge of neuromas now follows.

Neuromas

(i) Anatomy

Following the section of a peripheral nerve, degeneration occurs in the terminal parts of the severed axons of the proximal stump. The axons die back for a variable distance. This retrograde degeneration is usually confined to a few mm. in injuries that do not rupture the endoneurial tube, but in severe injuries the effects may extend proximally for several cm. (Young, 1942; Sunderland, 1978). The axons then emit sprouts. By 3-4 days after nerve section, sprouts are found in association with the majority of myelinated and unmyelinated axons (Aguayo, Peyronnard and Bray, 1973; Aguayo, Bray and Hopkins, 1975; Scadding, 1982; Scadding and Thomas, 1983). The sprouts are unmyelinated and often lie in close contact with one another, not separated by Schwann cell processes. More than 50 sprouts may be associated with a single axon (Weddell, 1942; Scadding, 1982).

One week after nerve section, many of the smaller sprouts and the terminal part of the parent axon have disappeared, the remaining sprouts being the larger diameter ones (Scadding, 1982). The outgrowth of sprouts advances distally towards the original point of injury. Two to three weeks after section, the terminal zone is composed of a mass of regenerating axons, fibroblasts, Schwann cells and blood vessels. The direction of

growth of the axons is influenced by the fibroblast-Schwann cell matrix which usually results in the axons being obstructed and deflected as they grow, (Guth, 1956). This leads to patternless growth, the axons crossing and intertwining, some growing in a retrograde direction, and the axons branch profusely (Sunderland, 1978).

The result of this chaotic growth is the conversion of the terminal part of the proximal nerve stump into a distinct swelling, known as a neurofibrogliomatous mass or neuroma (Young, 1942; Sunderland, 1978).

As a consequence of the amputation of a limb, every nerve trunk is severed. Each develops a neuroma, making the stump a site of multiple neuromas which vary in size and the degree to which they are exposed to pressure. As healing processes occur in the nerve ends and non-neural tissues at the same time, the neuromas tend to adhere to each other and to other tissues, i.e. skin, muscle, tendon bone or scar tissue. This means that movements of the stump can lead to traction on the neuroma (Sunderland, 1978).

The neuromas in amputation stumps tend to be larger than those observed after section of a peripheral nerve in an intact limb. This is because axonal growth is less restrained, and regenerating axons continue to escape from the neuroma into the

surrounding healing tissue. The axons can travel large distances from their source and they repeatedly encounter obstacles which deflect them, and they divide irregularly. This results in the stump tissues becoming reinnervated in a disorderly manner. Axon branching means that there is an increase in the density and complexity of the stump innervation (Sunderland, 1978).

(ii) Physiology

The physiological properties of the neuroma sprouts have recently been studied in detail by several workers, using an experimental "in vivo" neuroma preparation developed initially in the rat (Wall and Gutnick, 1974a,b; Devor and Wall, 1976). Briefly, this consists of either cutting and ligating the nerve, thus preventing the nerve sprouts from escaping the perineurium, or encapsulating the cut nerve end in a polythene tube with a sealed end, the neuroma forming within the tube. The experimenter can then perform electrophysiological recordings from the afferent fibres from the neuroma at any desired time after nerve section.

Results obtained from rat (Wall and Gutnick, 1974a,b; Govrin-Lippmann and Devor, 1978), mouse (Scadding, 1981) and cat (Blumberg and Janig, 1981, 1982a,b,1984) are in general agreement. They demonstrate that as the sprouts grow out, many

of the sensory fibres begin to generate nerve impulses. The number of fibres active increases with time, reaching a maximum at 1 to 3 weeks after nerve section in rats and mice (Govrin-Lippman and Devor, 1978; Scadding, 1981). After this there is a decline, so that after approximately 30 days there is a steady low level of afferent activity. This continues for at least 200 days (Devor, 1983). In cats the number of active fibres continues to increase up to 7 weeks after nerve section (Blumberg and Janig, 1984). Both myelinated and unmyelinated fibres have been shown to contribute to this activity (Blumberg and Janig, 1984). At the height of the spontaneous barrage, it has been calculated that approximately 50% of the myelinated sensory fibres are active, which represents a very large abnormal afferent input (Devor and Bernstein, 1982).

The firing patterns of the spontaneously active units have been reported as being of three types, either steady (with either a regular or irregular discharge) or phasic (firing cyclically in bursts) (Govrin-Lippmann and Devor, 1978; Scadding, 1981; Blumberg and Janig, 1984). The phasic firing pattern has been termed "interrupted autorhythmicity" (Wall and Devor, 1983).

(iii) Mechanosensitivity, Chemosensitivity and ephaptic connections

The neuroma afferents became mechanically sensitive as well as spontaneously active. Light pressure on the neuroma has been shown to increase the firing rate of a proportion of the spontaneously active fibres and to produce activity in previously silent fibres (Govrin-Lippman and Devor, 1978; Scadding, 1981; Blumberg and Janig, 1984).

Occurring at the same time as spontaneous discharge and mechanosensitivityof the neuroma sprouts, is the development of chemosensitivity. Adrenaline or noradrenaline administered intravenously or close-arterially results in an increased discharge in the neuroma afferents (Wall and Gutnick 1974a,b; Devor and Janig, 1981; Korenman and Devor, 1981; Scadding, 1981; Blumberg and Janig, 1984). A local rather than a systemic effect is indicated by the observation that the intravenous dose has to be about 5 to 10 times the close arterial dose for equivalent effects (Korenman and Devor, 1981). Reduced neuroma circulation produced by clamping the arterial blood causes increased firing rates followed by a cessation of discharge, but adrenaline does not act simply by causing local vasoconstriction as it can produce its effect if injected below an arterial clamp (Korenman and Devor, 1981).

Neuroma afferents were found to be sensitive to the

alpha-agonist phenylephrine at doses similar to the effective dose of adrenaline and noradrenaline, but failed to respond to the beta-agonist isoprenaline (Korenman and Devor, 1981). The effects of adrenaline were blocked by the alpha-blocker phentolamine but not by the beta-blocker propranolol (Devor and Janig, 1981). Firing of some fibres could also be affected by stimulation of the sympathetic trunk (Devor and Janig, 1981; Korenman and Devor, 1981).

It has been known for some time that the sympathetic nervous system plays a major role in pain resulting from some injuries, and that sympathetic block or sympathectomy can relieve this pain (Hannington-Kiff, 1974; Loh and Nathan, 1978; Bonica, 1979). The abnormal chemosensitivity of the neuroma sprouts provides a mechanism to explain this action of the sympathetic nervous system, i.e. excitation by circulating catecholamines and leaking catecholamines from damaged sympathetic fibres (Wall and Gutnick, 1974; Devor, 1983).

Another abnormal property of neuroma sprouts is the appearance of ephaptic connections between axons. This occurs in a low percentage of axons and consists of a tight electrical coupling such that impulses in one fibre can initiate an impulse in a neighbouring fibre (Selzer and Devor, 1979; Blumberg and Janig, 1981,1982a; Devor and Bernstein, 1982; Lisney and Pover, 1982, 1983). The overall effect of this would be to

enhance the abnormal afferent barrage in the damaged nerve.

Several possible mechanisms have been proposed to account for the abnormal excitability of axon sprouts in a neuroma. One of these is the proposition that the partial breakdown of the blood-nerve barrier in severed nerves could expose the neuroma fibres to an abnormal chemical and ionic environment which could alter their excitability (Devor and Bernstein, 1982). A recent suggestion is that an excess of current channels, particularly sodium and calcium, and alpha-adrenergic receptors are synthesised in the cell body and are axoplasmically transplanted to the axon sprouts where they produce abnormally high excitability and alpha-adrenergic chemosensitivity (Devor, 1983 ; Devor and Govrin-Lippmann, 1983).

Spontaneous afferent activity in the beak stump

The similarity between the discharge patterns of the spontaneously active units recorded from the chicken beak stump and those recorded from mammalian neuromas, and the fact that neuromas have been observed in the beak stump (Breward and Gentle, 1985) suggests that neuromas are the major source of the abnormal spontaneous activity in the chicken. Receptive fields, when located, were for the most part within 6 mm of the tip of the stump, with only a few (8) units located further

proximally (7-14mm). Units with proximal receptive fields did not have the properties of cold receptors. There are several possible explanations for the occurrence of abnormal spontaneous activity apparently originating at some distance from the neuroma. Firstly it is possible, although unlikely, that the effects of stimulation of the beak surface at points proximal to the neuroma could spread through epidermal and dermal tissue to the neuroma. Secondly, it is possible that neuroma sprouts growing in a retrograde direction were responsible for this afferent activity. A third possibility is that degenerating fibres proximal to the neuroma could be the source of the activity, as sites of demyelination in peripheral nerves can become a source of abnormal impulse generation (Howe, Calvin and Loeser, 1976; Howe, Loeser and Calvin, 1977; Burchiel, 1980, 1981; Calvin, Devor and Howe, 1982; Rasminsky, 1982, 1983). Degenerative changes following nerve section can be so extensive as to spread to the dorsal root ganglion and beyond. Interestingly, this seems to be particularly the case for the trigeminal nerve in mammals where tooth extraction can produce degeneration within the CNS (Gobel and Binck, 1977). These changes can induce abnormal spontaneous activity and mechanosensitivity in the dorsal root ganglion cells (Kirk, 1974; Weisenfeld and Lindblom, 1980; DeSantis and Duckworth 1982, Wall and Devor, 1983; Burchiel, 1984a,b).

What of the spontaneously active units, recorded from the beak

stump, with no receptive field? It is likely that this activity arose from neuroma sprouts or areas of demyelination that were protected, by surrounding tissues from external stimulation. Another possibility, although perhaps unlikely, is that this represented abnormal activity generated in the trigeminal ganglion, conducted antidromically along the nerve (Wall and Devor, 1983) to the neuroma and there reflected backwards, via ephaptic connections, to the point of recording.

A novel observation from the present study was the demonstration of thermal sensitivity of the spontaneously active units. The only other data on thermal sensitivity of abnormal spontaneously active afferents is that reported by Zimmermann and Sanders (1982) who recorded units from regenerating fibres after peripheral nerve crush. Three spontaneously active units were tested with cold (0°C) stimuli and excitation was produced in each case. Warming (35°C) was tested on one of these units, and produced an inhibition of discharge. In the present preparation a wide variety of effects were observed including some similar to those described by Zimmermann and Sanders (1982).

The relationship of the abnormal spontaneous afferent activity to sensation

In man, chronic pain syndromes, eg. causalgia and neuralgia, are associated with damage to peripheral nerves (Sunderland, 1978). Amputation of a limb leads to phantom limb sensation and sometimes phantom limb pain (Carlen, Wall, Nadvorna and Steinbach 1978; Wall, 1981). The prevalence of phantom limb pain after amputation can be quite high, e.g. Carlen et al reported that 67% of their patients reported phantom pain in the first few months. Two years after amputation 59% seen by Jensen, Krebs, Nielsen and Rasmussen (1985) were still experiencing phantom limb pain. The abnormal activity seen in peripheral nerves subsequent to nerve injury is thought to be a contributing factor to these abnormal sensations. Some direct evidence linking these two phenomena was obtained by Nystrom and Hagbarth (1981). Using the microneurographical technique, they recorded from nerves innervating the amputation stumps of two patients suffering from phantom limb pain. Abnormal spontaneously activity was recorded , and mechanical stimulation of the stump neuromas exacerbated the phantom limb pain and produced an afferent discharge. Blocking the neuromas with local anaesthetic abolished the mechanically-induced discharge and the exacerbation of the phantom limb pain. The spontaneous pain and the spontaneous afferent activity was not abolished by the block. This may have been due to the discharge arising from the dorsal root ganglion (Wall and

Davor, 1983).

In animals subjected to peripheral nerve section, it might be expected that any abnormal sensations experienced would result in changes in the animal's behaviour. It has been observed in mice, rats and monkeys that nerve section frequently leads to the animal attacking and mutilating the affected limb, a process termed autotomy (Wall, Devor, Inbal, Scadding, Schonfeld, Seltzer and Tomkiewicz, 1979; Wall, Scadding and Tomkiewicz, 1979; Inbal, Devor, Tuchendler and Lieblich, 1980; Wiesenfeld and Lindblom, 1980; Levitt and Levitt, 1981; Devor, Inbal and Govrin-Lippmann, 1982; Scadding, 1982). It has been suggested that autotomy is a manifestation of chronic abnormal sensations and possibly pain, and is related to the abnormal discharge from the neuromas. The precise nature of the relationship is the subject of current investigations. Devor and Raber (1983) have demonstrated that prevention of the development of neuroma discharge in myelinated fibres from a neuroma, using antimitotic drugs, did not prevent autotomy in rats. It is therefore possible that autotomy, at least in rats, is associated with the discharge generated in unmyelinated fibres, or from some other source of abnormal discharge, e.g. the dorsal root ganglion or to the CNS. In the present study, amputation of the beak of the chicken has been shown to result in abnormal discharges from the alveolar mandibular nerve. It would be expected that every nerve in both the upper and lower

beak that was severed in the process of beak trimming would undergo this process, leading to a large abnormal input to the CNS. Preliminary observations on the behaviour of chickens prior to and subsequent to beak trimming (Slee, Duncan and Breward, unpublished observations) have indicated that behavioural patterns can be altered by beak trimming. During the first three weeks after beak trimming, time spent feeding and drinking decreased and time spent dozing increased, compared to pre-operative values. These three activities returned to normal levels by five weeks after beak trimming. The time spent preening and pecking at the cage decreased and the time spent standing inactive decreased after beak trimming, and these values did not return to pre-operative levels for at least 5 weeks after beak trimming. The relevance of these observations to the abnormal afferent discharge and to possible sensations experienced by the bird obviously merits further investigation.

Chapter 5.

CONCLUSIONS

5. CONCLUSIONS

The hypothesis whch prompted the experiments described in this thesis was that beak trimming of the domestic fowl may cause pain and suffering to the bird. The theoretical and experimetal approach adopted in this thesis towards the study of pain perception in the chicken was to consider the first stage in the series of physiological events which lead to sensory perception, the response of cutaneous sensory receptors to external stimuli (Adrian , 1947; Zottermann, 1933, 1939).

Previous work in mammals has demonstrated the presence of a class of cutanous receptors which are selectively sensitive to acute noxious stimulation of the integument, nociceptors. The nociceptors are implicated in the transmission, from the periphery to the CNS, of information relating to the parameters of noxious stimulation (e.g. Perl, 1984). Although a substantial body of data regarding the physiological characteristics of mechanoreceptors, and to a lesser extent thermoreceptors, has accumulated for birds, there has been little previous systematic investigation of nociceptors and nociceptive mechanisms in this class of vertebrates. Cutaneous nociceptors have been physiologically characterized in the pigeon wing, but evidence for their presence in the chicken beak has not, until now, been conclusive.

The aims of the experimental work undertaken in the present investigation were: (i) To search for and characterize nociceptors in the beak of the chicken; (ii) To describe any differences in the primary afferent outflow from the trimmed beak compared to the intact beak. To this end, a new preparation was developed which allowed the electrophysiological recording of single unit afferent activity from the alveolar mandibular nerve innervating the lower beak.

The results described in Chapter 2 demonstrate the presence, in the intact beak, of cutaneous nociceptors. The characteristics of these nociceptors were similar to those of mammals. They were capable of encoding, in their afferent discharge, information relating to the temperature of the beak surface above the threshold of 41 °C. The process of beak trimming would be expected to stimulate these receptors. As the heated blade is brought close to the beak, increasing the surface temperature, the nociceptors would be excited. The first units to respond would be those with the lowest heat thresholds. As the temperature increases these would be driven towards their peak discharge in parallel with the activation of the units with progressively higher thresholds. Receptive fields were located all over the beak surface, hence information regarding the spatial location of the noxious stimulus would also be available to the CNS. It is likely that these nociceptor discharges represent the afferent arm of defense reflex

exhibited by the anaesthetized bird (Necker, 1977; Breward, unpublished observations) in response to noxious heating of the beak. Nothing, however, is known about the central processing of nociceptive information in birds.

The nociceptors present in the trimmed beak were similar to those in the intact beak and were also capable of coding the intensity of heat stimulation. The mean heat threshold of these nociceptors was not significantly different from that of the nociceptors in the intact beak, but their mean sensitivity and peak response were decreased compared to normal. These properties of the nociceptors did not provide a physiological basis for hyperalgesia and hyperpathia, contrary to the findings of Dickhaus et al. (1976a,b) in regenerating heat nociceptors in the cat.

A striking feature of the primary afferent outflow from the trimmed beak was the appearence of long lasting (up to at least 3 months) abnormal spontaneous discharges. Some of these discharges were modulated by cutaneous stimulation. They bore resemblance to the discharges observed from neuromas formed as the result of damage to mammalian peripheral nerves.

Beak trimming can therefore have both acute and chronic effects on the peripheral nervous system of the bird. These effects are similar to those described in other animals during acute

noxious stimulation of the integument and as a consequence of peripheral nerve injury. In man, these peripheral neural events can be accompanied by the subjective experience of acute and chronic pain. The direct verbal reporting of the subjective experience of pain is, of course, unique to man. Although there are numerous methodological problems involved in the assessment of pain in human beings (see Reviews by Hardy, Wolff and Goodell, 1952; Hardy, 1962; Melzack, 1983; Bromm, 1984; Chapman et al, 1985), an extra dimension of difficulty is faced in the assessment of pain in animals since there is no way of communicating directly with them. Logically, there is no reason to suppose that in evolutionary terms the perception of pain appears as a completely new sensory phenomenon in man, but the ascription of pain perception to animals remains an inference based on analogy.

Zimmermann (1985) has proposed the following definition of animal pain: "An aversive sensory experience that elicits protective motor actions, results in learned avoidance and may modify species-specific traits of behaviour, including social behaviour". The recognition of pain perception in animals is indirect, usually based upon the observation of physiological and/ or behavioural responses associated with pain in man, e.g. increased blood pressure, change in respiratory pattern, pupil dilatation, vocalization, and withdrawal movements. These responses can, however, be elicited in the decorticate

preparation and have been termed pseudo-affective reflexes (Sherrington, 1906). They are therefore not necessarily reliable indicators of conscious pain perception.

A perhaps more promising avenue of approach involves the analysis of behavioural responses which are more complex than simple reflexes. Several methods are available, e.g. operant conditioning (Casey, 1971; Duncan, 1985), motivational choice experiments (Chapman et al, 1985) and learned discrimination (Dubner et al, 1977). Duncan (1985) is of the opinion that operant conditioning procedures could be useful in assessing acute and chronic pain possibly experienced by farm animals as a result of operations such as beak trimming. The basis of operant conditioning is that animals will learn to perform a response in order to gain a reward or to avoid a punishment. If the assumption is made that the animal experience positive subjective feelings ("pleasure") in the presence of rewards and negative subjective feelings ("suffering" and/or "pain") in the presence of punishments, this technique could provide an objective means of assessing the subjective experiences of animals. Conditioned escape from a painful stimulus, i.e. a situation in which the animal learns to terminate or reduce the level of a painful stimulus, perhaps by self-administration of analgesia (e.g. Dib and Duclaux, 1982), could be a promising future approach to the study of the sensations perceived by farm animals as a result of operations on them. The animals

could be conditioned to respond to a standard painful stimulus and this response compared to that given during and after the operation.

The question of pain perception in animals, as well as providing an area of considerable scientific and philosophical involvement, has become the focus of intense debate in society as a whole, linked as it is with the problem of animal welfare (e.g. Dawkins, 1980). Beak trimming is one of a number of operations commonly carried out on farm animals (e.g. de-toeing, tail-docking, castration). The work described in this thesis has demonstrated that beak trimming can produce both acute and chronic responses from the peripheral nervous system of the domestic fowl. Similar responses are implicated in the perception of acute and chronic pain in man. Given this parallel the next logical step is to address the question of what the central nervous system of the chicken does with the information transmitted from the beak.

Several lines of approach for future work are apparent and are summarised below:

A. Peripheral Nervous System

The anatomical observations of Breward and Gentle (1985) should be extended, with particular reference to the longer term (>3 months) effects.

The electrophysiological observations on spontaneous afferent activity consequent to beak trimming should also be extended, with reference to both longer term effects and the possible involvement of the sympathetic nervous system. The possible involvement of the trigeminal ganglion in abnormal discharges should be pursued.

B. Central Nervous System

A pre-requisite to the study of the acute and chronic physiological effects of beak trimming on the CNS is the construction of a stereotaxic atlas of the brain stem of the adult hen. Anatomical observations on the trigeminal system of the chicken are necessary, and should be carried out with the appropriate pathway tracing techniques (e.g. degeneration studies, retrograde transport of horseradish peroxidase).

C. Behavioural

A behavioural model for the study of acute pain in the chicken is required. The use of operant conditioning procedures might be indicated here. The unpublished observations of Slee et al. (1985) on the chronic effects of beak trimming should be followed up. Ultimately a correlative electrophysiological/behavioural study using the awake unrestrained animal would be desirable.

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Cutaneous nociceptors in the chicken beak

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To prevent feather pecking and cannibalism, intensively reared chickens are subjected to 'beak trimming' (i.e. amputation with a heated blade of the anterior one half to one third of the beak) but objections to its practice have been raised on welfare grounds. The only information available on the cutaneous afferent innervation of the chicken beak comes from a preliminary study by Roumy & Leitner (1973). Using electrophysiological techniques, they demonstrated the presence of cold receptors, rapidly adapting mechanoreceptors and high-threshold slowly adapting mechanoreceptors. The purpose of the present experiments was to search for and characterize cutaneous afferents which respond to noxious stimulation.

Adult Brown Leghorn hens were anaesthetized with urethane (25%, I.V.), a wing vein was cannulated and a tracheotomy performed. Body temperature was maintained at 40 °C with a homoeothermic blanket. The intramandibular nerve, which supplies the cutaneous afferent innervation to the lower beak, was exposed in the mandibular canal. The nerve was supported on a black Perspex platform in a pool of liquid paraffin. Recordings were made from single fibres in fine filaments dissected from the nerve. Von Frey hairs and a feedback-controlled radiant-heat stimulator were used to determine mechanical and thermal thresholds, response characteristics, and to map receptive fields. Conduction velocities were determined by electrical stimulation of the receptive field.

Ten single units were isolated that responded to high-stimulus temperatures and to mechanical stimulation of the receptive field. None of the units was active at normal beak temperatures and none responded to cooling. Thermal thresholds ranged from 40 to 48 °C (mean 43·0, S.D. 3·5) and mechanical thresholds ranged from 1·4 to 50 g. A persistent discharge was evoked during sustained suprathreshold mechanical or thermal stimulation of these units. In the case of thermal stimulation, the discharge increased with increased temperature. The receptive fields, circular or oval in shape and with diameters of 1–5 mm, were distributed evenly over the external beak surface, down to the beak tip. They were found to be uniformly sensitive to mechanical stimulation. Conduction velocities, measured for six units, ranged from 0·53 to 1·85 m/s (mean 0·95, S.D. 0·48), corresponding to unmyelinated axons.

These results demonstrate the presence in the chicken beak of cutaneous afferents which respond to potentially tissue-damaging thermal stimuli and hence may be termed nociceptors, in common with similar afferents found in mammals (Iggo, 1977).

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Neuroma formation and abnormal afferent nerve discharges after partial beak amputation (beak trimming) in poultry

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Summary. Following partial amputation of the beak recordings were taken of the electrical activity from single afferent fibers of the intramandibular nerve. A total of 192 single afferent fiber units were isolated of which 67 were classified as nociceptors, with an abnormal pattern of discharge, and 89 were abnormal spontaneously active units. Following amputation neuromas were developing by 15 days after surgery and they were formed by 20 to 30 days. The presence of neuromas together with abnormal spontaneous activity originating from them raise serious welfare questions concerning beak trimming.
Key words. Poultry; beak trimming; intramandibular nerve; neuroma; afferent electrical activity.

The mutilation of farm animals to prevent them developing damaging behavioural vices is an emotive subject but there is little scientific evaluation of these in relation to the animal's welfare. Partial amputation of the beak is often performed in laying and breeding chicks and sometimes in adult birds to prevent or control cannibalism and feather pecking. Removal of part of the beak stimulates nociceptors[1] and creates feeding difficulties[2].

Previous studies of peripheral nerve injury and subsequent neuroma formation in the mouse and rat have suggested that abnormal activity arising from the regeneration axons is implicated in post-amputation stump pain[3]. However, with the exception of one clinical report[4], no experimental work has been performed on amputated stumps. The beak of the chicken is extensively innervated and has numerous cutaneous sensory receptors which have been classified as low-threshold mechanoreceptors, cold receptors and nociceptors[1,5]. These nociceptors have properties very similar to those described in mammals including man[6–12] and they are excited during the process of beak trimming. It is, however, not known what kind of information the damaged beak conveys to the central nervous system. The aim of this study was to investigate the long-term neurological consequences of beak trimming by examining both anatomically and physiologically the nerves running to the stump of the beak.

Methods. Adult Brown Leghorn hens, weighing from 1 to 1.5 kg, were anesthetized with sodium pentobarbitone (Sagatal, May & Baker Ltd) given i.v. The response of individual birds to barbiturate anesthesia is variable so that the level of anesthesia was monitored using the comb-pinch reflex (dose 24–30 mg). About ⅓ of the upper and lower beaks were removed using a commercial heated blade debeaker (Cope & Cope Ltd). The debeaker consisted of a lower metal bar on which the beak was placed and a movable electrically heated upper blade. The upper blade is manually pushed against the beak where it cuts through the beak cauterizing the stump at the same time. At intervals ranging from 1 to 83 days after beak trimming they were anesthetized (Ethyl carbamate, 1.5 g/kg given i.v.) and recordings were made from single afferent fibers of the intramandibular nerve. The intramandibular nerve, which supplies the cutaneous afferent innervation of the lower beak, was exposed in the mandibular canal. The nerve was supported on a black perspex platform in a pool of liquid paraffin. Fine filaments of the nerve, containing single active fibers, were dissected using fine watchmakers forceps and placed over silver wire recording electrodes.

To investigate the anatomical consequences of beak trimming 20, 5-week-old, Brown Leghorn chickens were anesthetized (sodium pentobarbitone) and ⅓ of the upper and lower beak was removed in the same way as the birds used for the electrophysiology. Two birds were killed at various intervals after beak trimming (1, 3, 6 and 24 h and 3, 6, 10, 15, 20, 30 days), the beaks were removed and fixed in formalin-acetoalcohol. The tissue was decalcified, embedded in paraffin wax and serially sectioned at 10 µm. All sections were collected and the slides were stained with either Hematoxylin/Eosin or Bodian's Protargol or Masson's Trichrome.

Results and discussion. A total of 192 single afferent fiber units were successfully isolated from 36 beak-trimmed birds. 28 of these units were classified as low threshold mechanoreceptors[5,13], with properties similar to those encountered in the intact beak.

67 units were classified as nociceptors[1], differing only slightly from normal nociceptors in thresholds and stimulus-response properties, but showing an abnormal pattern of discharge. Normal nociceptors exhibited a persistent discharge during sustained suprathreshold mechanical or thermal stimulation and in the case of thermal stimulation the discharge increased with increased temperature. Thermal thresholds of normal nociceptors ranged from 40° to 48 °C (mean 43.0, SE 0.66) and mechanical thresholds from 1.4 to 50 g^1. Unlike the regenerating C-heat receptors reported in the cat[14] there was no systematic reduction in thermal thresholds in the nociceptors found in the amputated stump (thresholds 36–62 °C, mean 47.0, SE 0.89). In the intact beak the nociceptors responded to a suprathreshold thermal stimulus with a continuous train of impulses[1] with only 6% of the units showing a bursting (grouped) discharge. Whereas 37% of the nociceptors recorded from the amputated stump responded with a bursting pattern of discharge. The mechanoreceptors and nociceptors present in the amputated beak stump, like those in the intact beaks, did not discharge in the absence of stimulation.

The most characteristic abnormality encountered in the beak stump was the presence of large numbers of spontaneously active units. A total of 89 such units were recorded, 53 of which are reported here. For each of these 53 units their receptive fields were located and the unit was tested for responsiveness to heat, cold and mechanical stimuli using the techniques described previously[1]. The pattern of spontaneous discharge was basically either regular, irregular or bursting (fig. 1). The regular and irregular patterns in some units had occasional periods of bursting superimposed on the basic discharge pattern. The effects of heat, cold and mechanical stimulation on the rate of response varied from unit to unit. There were eight classes of stimulus-modality responses (table) ranging from an excitatory effect (i.e. increase in discharge rate) to all three modalitites (heat, cold, mechanical) to total unresponsiveness to any stimulus. Three spontaneously active units had response characteristics similar to cold receptors i.e. a regular spontaneous discharge which was excited by a cold stimulus, inhibited by heat and unaffected by mechanical stimulation. The remaining 50 units were completely abnormal in their discharge and response characteristics. They were recorded from the beak stump at 5–83 days after the initial amputation with their receptive fields located on the distal tip of the stump and at varying distances (up to 12 mm) proximal to it. This spontaneous activity is markedly similar to that observed in the experimental neuroma preparation developed initially by Wall, Devor and coworkers[15–17] in the rat and later extended to the mouse and cat[18,19].

Beak trimming results in both cutting and cauterizing the beak, and a significant but variable amount of the remaining beak was damaged by the cautery. The nerves in the beak were damaged by the high temperature of the cautery blade for a distance of 2–3 mm from the cut end. By 6 days after trimming the damaged portion of the nerve had been completely degenerated. At 10 days there was evidence of nerve regrowth with some enlargement of the end of the nerve. This regeneration and regrowth of the nerve fibers continued so that by 15 days clear neuroma was present at the end of the nerve stump together with numerous bundles of regenerating fibers. These regenerating fibers continued to grow but, because of the adjacent scar tissue, were unable to innervate dermal structures and consequently the fibers grew back on themselves to form a complex mass of intertwining regenerating nerve fibers together with the surrounding tissue.

In some nerves there was a simple terminal neuroma. In other a neuroma was formed at the original stump of the nerve (fig. 2A, B) in association with a large and complex neuroma formed adjacent to the scar tissue which forms the end of the beak. Some nerves did not appear to have a neuroma at the original stump but, instead, had a complex neuroma adjacent to the scar tissue (fig. 2C, D).

The activation of specific nociceptors in humans[20] and spontaneous discharges originating from stump neuromas are implicated in acute and chronic pain syndromes. From previous work[1] it is clear that the process of beak trimming results in the activation of specific nociceptors in the beak at the time of surgery. From

the work presented here it is clear that neuromas are formed as a result of the amputation and that these neuromas probably give rise to abnormal spontaneous nervous activity.

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Figure 1. Three examples of afferent spontaneous activity recorded from the intramandibular nerve of the amputated beak. *a* Unit with a regular (9 Hz) discharge pattern, recorded 36 days after amputation. The receptive field was located 1 mm proximal to the tip of the amputation stump. *b* Unit with an irregular discharge pattern, recorded 37 days after amputation. The receptive field could not be located using heat, cold or mechanical stimulation. *c* Unit with a bursting discharge pattern, recorded 31 days after amputation. The discharge pattern within each burst is regular. The receptive field was located 3 mm proximal to the tip of the amputation stump. Time calibration bar = 1 s in *a* and *c*, 5 in *b*.

Figure 2. Photomicrographs of 10 μm thick sections of the upper beak of the chicken 30 days after beak trimming. All sections stained with trichrome method. *A* Lower power (× 100) section showing a neuroma formed at the stump of the nerve (SN) with a complex mass of regenerating fibers (R) growing towards the scar tissue (ST) at the end of the beak. *B* A higher magnification of the stump neuroma (× 400) shown in *A*. *C* Low power (× 100) section showing regenerating fibers forming a complex neuroma (N) adjacent to the scar tissue at the end of the beak. *D* A higher magnification (× 400) of the neuroma shown in *C*. E = epidermis, D = dermis, B = maxilla bone.


a
b
c

Spontaneously firing units recorded from the intramandibular nerve of beak-trimmed chickens. The ongoing activity was recorded, then each unit was tested for its responsiveness to heat, cold and mechanical stimulation. 53 units were successfully isolated; they are divided into eight groups on the basis of their responsiveness to the different stimulus modalities

Number of units	Stimulus			Spontaneous firing pattern	Time after operation (days)	RF location (mm)
	Heat	Cold	Mechanical			
3	↑	↑	↑	I, B, I + B	5–38	2–12
5	↑	N.E.	↑	I, I + B	7–30	0–11
1	↑	N.E.	N.E.	B	22	7
1	N.E.	↑	N.E.	I	31	0
25	N.E.	N.E.	N.E.	R, I, I + B, B	13–83	N.L.
11	↓	↑	N.E.	R.I.	6–83	0–4
6	↓	↑	↑	I, R + B	12–83	0–4
1	↓	↑	↓	B	31	3

↑ = Stimulus provokes an increase in the firing rate of the unit. ↓ = Stimulus provokes a decrease in the firing rate of the unit. N.E. = Stimulus has no effect on the firing rate of the unit. R = Regular firing pattern. I = Irregular firing pattern. B = Bursting firing pattern. R + B = Regular firing pattern with occasional bursting. I + B = Irregular firing pattern with occasional bursting. Receptive field (RF) location refers to the distance (measured to the nearest 0.5 mm) from the distal end of the beak stump to the distal edge of the receptive field. N.L. = Receptive field not located.

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The bill tip organ of the chicken (*Gallus gallus* var. *domesticus*)

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INTRODUCTION

Complex arrangements of mechanoreceptors in the avian beak have been reported in a variety of species (Goglia, 1964; Bolze, 1969; Gottschaldt & Lausmann, 1974; Krulis, 1978; Berkhoudt, 1976, 1980). In the chicken, Gottschaldt & Lausmann (1974) reported the presence of 15–20 horny tubules in the lower beak but no similar structures were noted in the upper beak. Although Desserich, Fölsch & Ziswiler (1984) have made a systematic study of Herbst and Merkel corpuscles in the beak of the chicken before and after partial beak amputation, they make no specific mention of specialised beak tip structures. They report, however, that Merkel corpuscles can be found in particularly great numbers in the corium papillae of the beak tip.

These specialised dermal papillae with different kinds of receptors have features which are similar to many of the complex sensory structures found in mammals (Iggo & Gottschaldt, 1974).

In the goose it has been suggested that the presence of these dermal papillae may enable a higher resolution of tactile sensory information (Gottschaldt & Lausmann, 1974) and a similar argument has been put forward by Krulis (1978). In the mallard (Zweers & Wouterlood, 1973), woodcock & snipe (Goglia, 1964), and probably in many other species, food discrimination occurs at the level of the beak tip organ. The complex arrangements of mechanoreceptors in the beak of the Fringillidae are clearly correlated with the highly complex seed-husking mechanism of this group (Krulis, 1978).

There has, however, been no detailed account of the specialised dermal papillae found in the lower beak of the chicken. Since partial amputation of the beak (beak trimming) is a common agricultural practice, it is important to study these specialised sensory structures in detail so that the sensory deficits following their damage or removal can be fully evaluated.

MATERIALS AND METHODS

The surface features of the lower beak were studied using the scanning electron microscope. The lower beaks of three newly hatched chicks, which had been killed previously, were removed, mounted and gold sputter coated using a Polaron E 5100 Sputter Coater. The surface features of the beak were then examined using a Cambridge Stereoscan 180.

To investigate the general structure of the beak tip histologically, 5 weeks old birds were used. Fifteen birds were killed, the lower beak was removed and fixed for

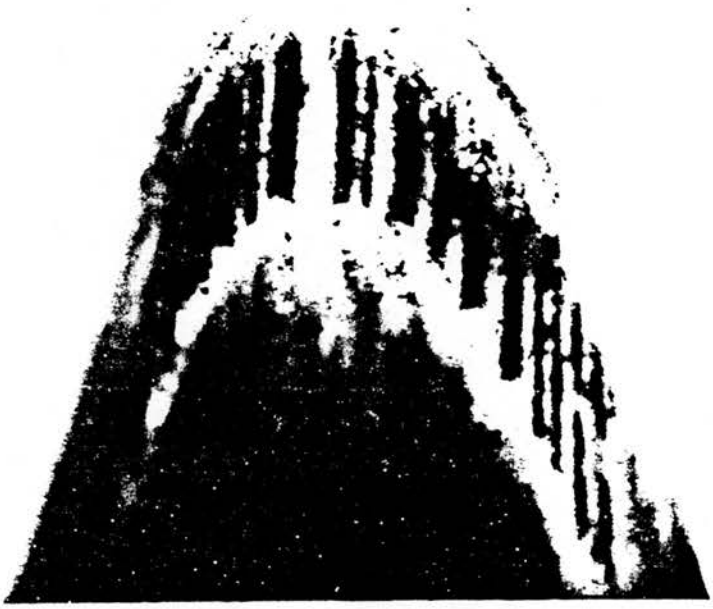


Fig. 1. The tip of the lower beak of a 5 weeks old bird when viewed from above using transmitted light. The dermal papillae can be clearly seen. Calibration bar, 0·5 mm.

2 weeks in 10% formol saline. Following fixation, they were decalcified in a 10% formic acid formalin solution for 2 weeks and washed in running water overnight. The outer keratin of the beak proved to be very difficult to section and in some beaks it was removed mechanically with a scalpel before further processing. In other beaks, the keratin was softened with a solution of 4% calcium thioglycolate in water for 2 weeks. Following washing the beaks were dehydrated in three changes of dioxan, vacuum embedded in Fibrowax (P. A. Lamb) and serially sectioned (longitudinally) at a thickness of 10 μm. The sections were stained with either haematoxylin and eosin, Bodian's protargol or Masson's trichrome.

RESULTS

The presence of 15–20 dermal papillae in the tip of the flower beak which had been reported by Gottschaldt & Lausmann (1974) was confirmed. These could be seen clearly when the lower beak was viewed from above using transmitted light (Fig. 1). No comparable structures were seen in the upper beak.

The surface features of the inside of the lower beak are shown in Figure 2. There is a single row of oval, shallow pits situated just inside the mouth caudal to the cutting edge (tomial edge) of the beak. These surface pits corresponded to the ends of the dermal papillae. There was no suggestion of any specialised tactile structures in or adjacent to these pits.

Low magnification photomicrographs of sections taken at the end of the lower beak with the keratin intact (Fig. 3) showed the finger-like dermal and epidermal projections into the thick keratin (the rhamphotheca) of the exterior of the beak. The epidermal cells in these papillae extended to the periphery and, although the

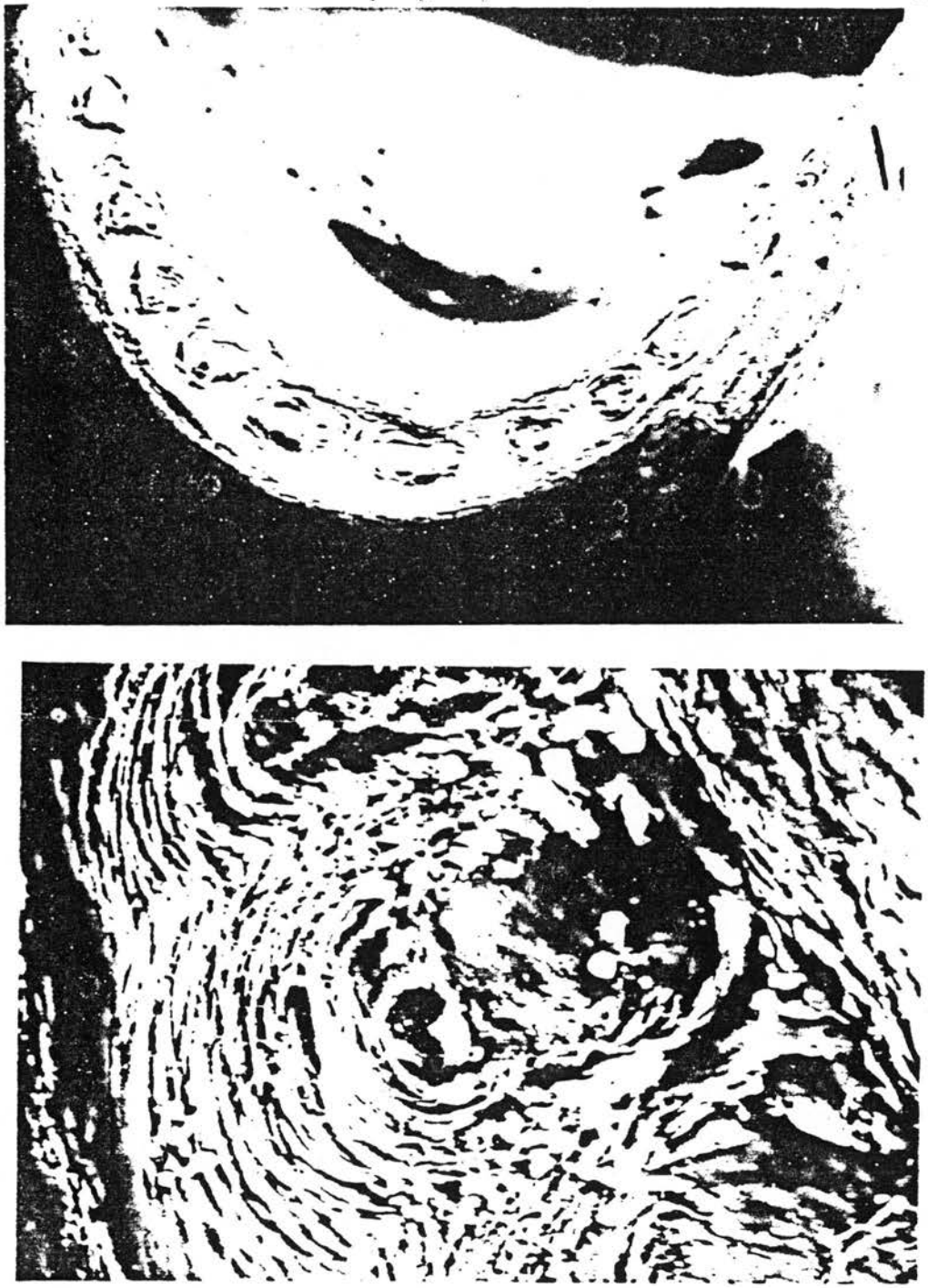


Fig. 2(A–B). Low magnification scanning electron micrograph of the beak tip of a newly hatched chick. × 34. (B). A higher magnification scanning electron micrograph of the shallow pit situated on the beak just inside the mouth. × 396.

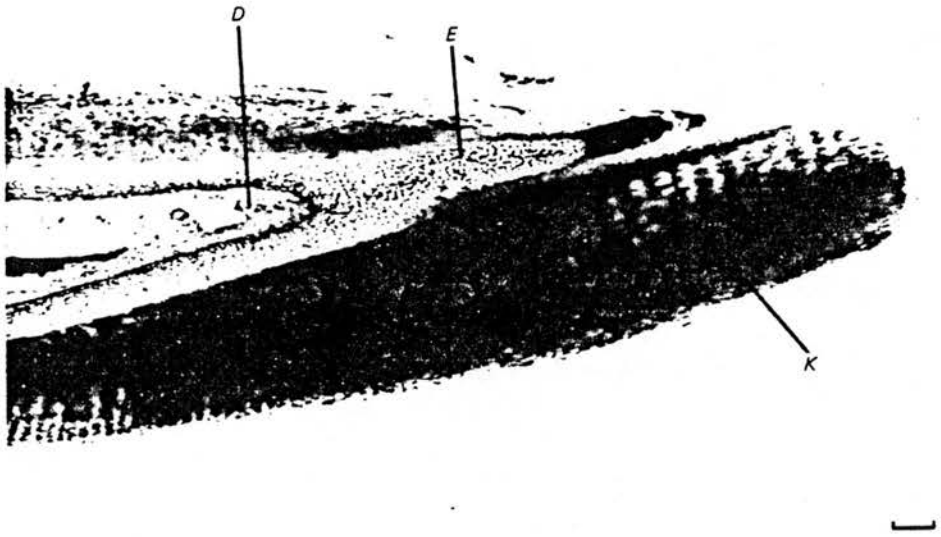
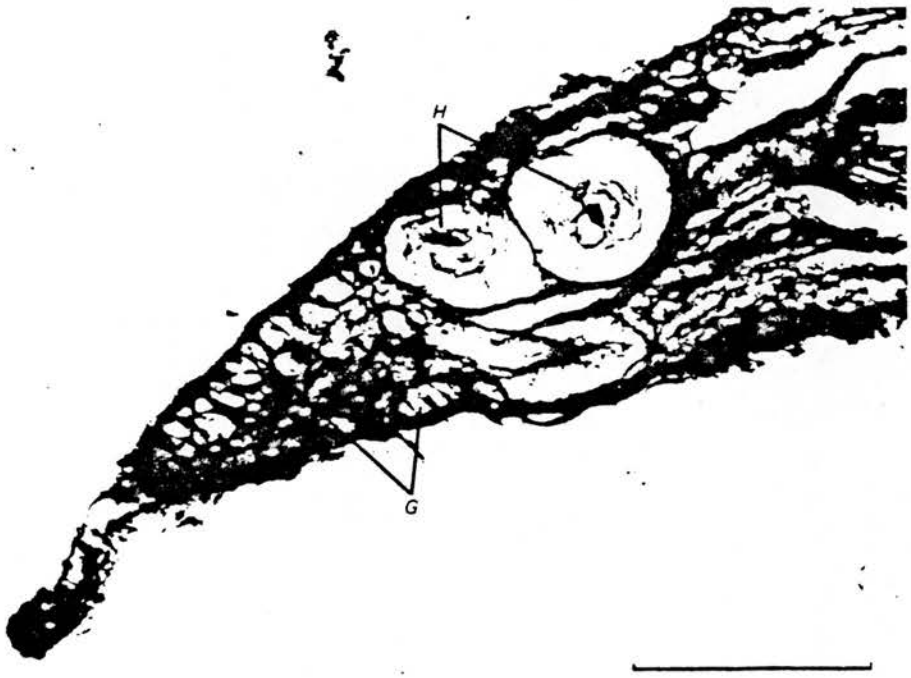


Fig. 3. Low magnification photomicrograph of a 10 μm thick section of the tip of the beak stained with Bodian's protargol showing the finger-like dermal (*D*) and epidermal (*E*) projection through the thick outer keratin (*K*) of the beak. Calibration bar, 100 μm.

nuclei became flattened, the degree of keratinisation was less extensive than that seen in the surrounding rhamphotheca. The dermis extended some distance into the papillae and contained numerous blood vessels and nerve fibres. At the base of the papillae, there was a cluster of Herbst corpuscles. Usually three Herbst corpuscles were present in each papillae (Fig. 4) but in some papillae five corpuscles have been seen. Because of the closeness of the papillae and the large size of the Herbst corpuscles, estimates for their number per papilla were difficult to obtain. In the distal parts of the papillae, there were large numbers of corpuscles of the Merkel (Grandry) type. These were orientated within the papillae at right angles to their normal orientation within the dermis. In the dermis, Merkel corpuscles were often found directly below the epidermis with the longitudinal axis of the individual tactile cells aligned parallel to the surface of the epithelium.

DISCUSSION

The dermal papillae of the beak tip of the chicken show many structural similarities to the bill tip organ of the duck (Berkhoudt, 1976; 1980) and the goose (Gottschaldt & Lausmann, 1974): there are large numbers of Merkel (Grandry) corpuscles in the distal region of the papillae and Herbst corpuscles in the proximal region. The Merkel corpuscles of the chicken and quail have numerous similarities with the Grandry corpuscles of chicks and geese (Anderson & Nafstad, 1968; Nafstad, 1971; Saxod 1978). Ide & Munger (1978), on the basis of morphological, physiological and embryological evidence, have proposed the term 'Grandry corpuscles' for all avian dermal sensory corpuscles containing the characteristic Grandry cells. Confusion in



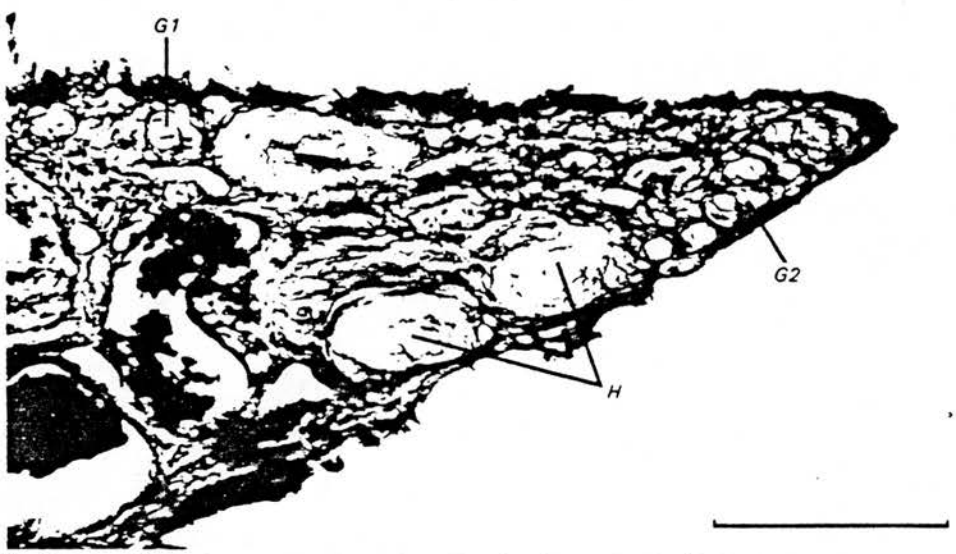


Fig. 4(A–B). Photomicrographs of two dermal projections stained with Bodian's protargol. (A) This shows Herbst (*H*) and Grandry (*G*) corpuscles. (B) The difference in orientation of the Granetry corpuscles can be seen with those at the base of the papillae (*G1*) showing their normal epithelia orientation, i.e. longitudinal axes parallel to the surface, whereas those in the papillae (*G2*) are at right angles to it. Calibration bar, 100 μm.

terminology, however, still arises and Desserich *et al.* (1984), working on the chicken, have recently reported the presence of Merkel corpuscles but state that they cannot find any Grandry corpuscles.

Both the Herbst and the Grandry corpuscles are considered to be very sensitive, rapidly adapting mechanoreceptors (Dorward, 1970; Gottschaldt, 1974; Gregory 1973; Leitner & Roumy, 1974; Leitner, Roumy & Saxod, 1973). The free nerve endings observed by Desserich *et al.* (1984) are likely to respond to thermal stimulation and noxious stimulation (Breward, 1984) and to give responses to prolonged maintained displacement. Perhaps the reduced keratinisation of the cells in the tip of the papillae permits a greater displacement of the softer tissue than the surrounding rhamphotheca and would allow for an increased sensitivity. The large numbsr of mechanoreceptors in the papillae suggests that, as with many other birds, the chicken has developed these specialised structures in the tip of the beak to provide the necessary fine tactile discrimination to enable them to perform a number of complex oral tasks. The absence of these specialised dermal papillae in the upper beak are of considerable interest. Although dermal papillae, Merkel (Grandry) and Herbst corpuscles are all present in the upper beak, they are not arranged into specialised papillae.

The papillae being so close to the most distal point of the beak, partial amputation, however, sligyt, must lead to a considerable loss of sensory input which may be reflected in the feeding difficulties shown by the birds after this procedure (Gentle, Hughes & Hubrecht, 1982). Breward & Gentle (1985) have provided evidence for both acute and chronic pain followed beak trimming, though it is not certain how these relate to loss of the specialised sensory organs.

SUMMARY

At the tip of the lower beak of the chicken there were found 15–20 specialised dermal papillae containing large numbers of mechanoreceptors. The Merkel or Grandry corpuscles were situated distally in the papillae and at the papillae base was a collection of Herbst corpuscles. The apex of the papillae, under the scanning electron microscope, appeared as a row of shallow pits on the surface of the beak just inside the mouth. These papillae resemble similar structures seen in other birds and are probably necessary for fine tactile discrimination.

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